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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<p>(51) International Patent Classification 5 : A61K 37/02, 39/00, C07K 7/08 C07K 7/10, 13/00, 15/00 C07K 15/28</p>	<p>A1</p>	<p>(11) International Publication Number: WO 91/04745 (43) International Publication Date: 18 April 1991 (18.04.91)</p>
<p>(21) International Application Number: PCT/US90/05105 (22) International Filing Date: 13 September 1990 (13.09.90) (30) Priority data: 413,332 27 September 1989 (27.09.89) US 571,267 23 August 1990 (23.08.90) US (71) Applicant: ATHENA NEUROSCIENCES, INC. [US/ US]; 800F Gateway Boulevard, South San Francisco, CA 94080 (US). (72) Inventors: RUBIN, Lee, L. ; 122 Funston Avenue, San Francisco, CA 94118 (US). LIAW, Chen, W. ; 80 Maria- na Court, Redwood City, CA 94062 (US). TOMASELLI, Kevin, J. ; 485 Vallejo Street, San Francisco, CA 94133 (US).</p>		<p>(74) Agents: BLECHER, Melvin et al.; 611 West Sixth Street, 34th Floor, Los Angeles, CA 90017 (US). (81) Designated States: AT (European patent), BE (European patent), CA, CH (European patent), DE (European pa- tent)*, DK (European patent), ES (European patent), FR (European patent), GB (European patent), IT (Euro- pean patent), JP, LU (European patent), NL (European patent), SE (European patent). Published <i>With international search report. Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i></p>
<p>(54) Title: COMPOSITIONS FOR CELL ADHESION INHIBITION AND METHODS OF USE</p>		
<p>(57) Abstract</p> <p>Compositions that disrupt microvascular endothelial and epithelial cell tight junctions, and methods of use, are disclosed. Such compositions comprise agents that inhibit the binding to such cells of cell adhesion molecules. Such inhibitor agents include cell adhesion molecules, fragments of cell adhesion molecules that encompass a cell-binding domain such as HAV, and antibodies directed against cell adhesion molecules and fragments thereof. Also disclosed are drug delivery compositions comprising a therapeutic drug conjugated to an agent that disrupts cell tight junctions.</p>		
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**COMPOSITIONS FOR CELL ADHESION INHIBITION
AND METHODS OF USE**

This is a continuation-in-part of United States
Serial No. 07/413,332, filed September 27, 1989.

5 **Background of the Invention**

Field of the Invention

 This invention relates to compositions that
transiently and reversibly dissociate the blood-brain
barrier. More particularly, the invention relates to
10 compositions that dissociate tight junctions between
brain capillary endothelial cells that constitute the
physiological barrier between the general circulation
and the brain.

Detailed Description of Related Art

15 The entry of drugs from the blood stream to the
central nervous system (CNS), i.e., the brain and
spinal cord, is restricted by the presence of high
resistance tight junctions between brain capillary
cells and by the apparently low rate of transport
20 across these endothelial cells (Betz, A.L., et al.,
Ann. Rev. Physiol., 48:241 (1986); Pardridge, W.M.,
Ann. Rev. Pharmacol. Toxicol., 28:25 (1988)).

 The tight junctions of the blood brain barrier
(BBB) prevent diffusion of molecules and ions around
25 the brain capillary endothelial cells. The only
substances that can readily pass from the luminal core
of the capillary to the abluminal tissues that surround
the capillary are those molecules for which selective
transport systems exist in the endothelial cells, as
30 well as those compounds that are lipophilic (i.e.,
hydrophobic). In contrast, drugs, peptides and other

molecules that are neither lipophilic nor transported by specific carrier proteins are barred from entry into the brain, or their rates of entry are too low to be useful, thereby imposing a severe limitation upon the physician's ability to treat CNS disorders pharmacologically.

The carrier-mediated transcellular transport system mentioned above may have limited usefulness for therapeutic modalities under some circumstances.

Transcytotic transport, in general, involves, first, the binding of molecules to specific carrier proteins on the surface of endothelial cells, and, second, the delivery of such molecules across the endothelial cells. Limitations on the usefulness of such a system for treatment of CNS disorders are based on the following considerations: (1) physiological carrier proteins may not function efficiently, or at all, with non-physiological drugs; (2) even where function occurs, the rate of transport of therapeutic agents will be limited by the rate of transport of the carrier; (3) the overall capacity of cerebral capillary endothelial cells to transport any therapeutic macromolecules may be simply too low to achieve therapeutic levels of certain drugs in the brain; and (4) once therapeutic macromolecules enter endothelial cells, depending on their nature, they might be delivered to any number of organelles, including lysosomes that contain a wide variety of hydrolytic enzymes. For these reasons, creating drug delivery systems that do not rely upon transcytosis will clearly be advantageous.

As tight junctions between brain capillary endothelial cells constitute a major part of the BBB, the possibility of modifying these junctions has been considered. It has been found that tight junctions,

including those of the BBB, can be disrupted by hyperosmotic solutions administered intra-arterially. For example, Polley et al., WO89/04663, published June 1, 1989, disclose the osmotic disruption of the interendothelial structure of the BBB by the intra-arterial administration of hypertonic solutions of mannitol, arabinose or glycerol as a means of introducing into the brain genetic material. Similarly, hyperosmotic solutions of urea have also been used to alter the BBB (Bowman, P.D. et al., Ped. Res., 16:335A (1982)).

Other chemical agents have been reported to disrupt endothelial or epithelial cell tight junctions when administered intravenously, including:

7-fluorouracil (MacDonell, L.A., et al., Cancer. Res., 38:2930 (1978)), degradation by membrane enzymes (Vincent, P.A., et al., Exp. Mol. Path., 48:403 (1988); Diener, H.M., et al., J. Immunol., 135:537 (1985)), aluminum salts (Zigler, Z.Y., et al., IRCS Med. Sci., 12:1095 (1984)), histamine (Meyrick, B., et al., Exp. Lung Res., 6:11 (1984)), thrombin (Siflinger-Birnboim, A., et al., Microvasc. Res., 36:216 (1988)), phorbol esters (Shiba, K., et al., Exp. Cell Res., 178:233 (1988)), and neutralization of the luminal anionic charge (Hart, M.M., J. Neuropathol. Exp. Neurol., 46:141 (1987)).

Although the above-listed modalities may disrupt tight junctions and thereby increase permeability of the BBB, problems attendant upon their use make them less than desirable. For example, intra-arterial perfusion with hyperosmotic solutions involves surgery, and this cannot be repeated on a regular basis. Further, concentrated sugar solutions may not be innocuous, and might be expected to have undesirable side effects. In addition, the aforementioned chemical

agents may not be useful for the treatment of chronic neurological disease, their effects on tight junctions are not always reversible, and, as they all are themselves powerful drugs, there is always the danger
5 that their use will compromise the patient's health generally. For example, 7-fluorouracil is a powerful inhibitor of pyrimidine synthesis, and thus nucleic acid biosynthesis, in animals cells.

Thus, an important need still exists for means
10 which transiently and reversibly disrupt tight junctions of the BBB in order that administered drugs can reach the brain from the general circulation, and which have no undesirable side effects of their own in the subject.

15 Attempts have been made to disrupt cell-cell adhesion by modifying the protein(s) responsible for such adhesion, collectively referred to as "cell adhesion molecules" (CAM). One class of CAM is termed "cadherin". "Cadherin" is the term applied to a family
20 of glycoproteins found in most kinds of mammalian tissues and thought to be responsible for Ca^{2+} -dependent cell-cell adhesion, (Takeichi, M., Development, 102:639 (1988)). Three subclasses of cadherin have been identified, namely, E-cadherin (from
25 epithelial tissues), P-cadherin (from placental tissues), and N-cadherin (from neural tissues) (Yoshida-Noro, C., et al. Dev. Biol., 101:19 (1984); Nose, A., et al., J. Cell Biol., 103:2649 (1986); Hatta, K., et al., Nature, 320:447 (1986)).

30 The different cadherins exhibit distinct tissue distribution patterns (Takeichi, U., (1988) above). E-cadherin, which was found to be distributed exclusively in epithelial cells of various tissues (Hatta, K., et al., Proc. Nat'l. Acad. Sci. (USA),
35 82:2789 (1985); Takeichi, 1988, above), appears to be

identical to uvomorulin (Hyafil, F., et al., Cell, 21:927 (1986)), chicken liver-cell adhesion molecule (L-CAM, Gallin, W.J., et al., Proc. Nat. Acad. Sci. (USA), 80:1038 (1983)), and cell-CAM 120/80 (Damsky, C.H., et al., Cell, 34:455 (1983)) in terms of biochemical properties (Cunningham, B.A., et al., Proc. Nat. Acad. Sci. (USA), 81:5787 (1984)) and tissue distributions (Thiery, J.-P., et al., Dev. Biol., 102:61 (1984)).

10 N-cadherin, which is expressed in various neural tissues including astrocytes (Hatta, K., et al., Devel. Biol., 120:215 (1987); Matsunaga, M., et al., Nature, 334:62 (1988); Tomaselli, K.J., Neuron, 1:33 (1988)), shows 92% amino acid sequence homology between
15 mammalian and avian homologs, shows from 40 to 50% similarity to epithelial E-cadherin and to placental P-cadherin of the same species, but was immunologically not cross-reactive with other cadherins within the same animal (Miyatani, S., Science, 245:631 (1989)).

20 Placental P-cadherin has also been cloned, and the deduced amino acid sequence of this glycoprotein was found to exhibit about 58% homology with epithelial E-cadherin (Nose, A., et al., EMBO J., 12:3655 (1987)).

Subsequent to the September 27, 1989 filing of the
25 parent application, Heimark, et al. (Heimark, R.L., et al., J. Cell Biol., 110:1745 (1990) reported on the identification of a Ca^{2+} -dependent cell-cell adhesion molecule in aortic endothelial cells.

Although each of the aforelisted cadherins
30 displays unique immunological and tissue distribution specifications, all have features in common: (1) a requirement for Ca^{2+} for cell adhesion function; (2) protection by Ca^{2+} from proteolytic cleavage; (3) similar numbers of amino acids, i.e., from about 723 to
35 about 822; (4) similar masses, i.e., about 124 kdal.

for the glycoprotein; (5) substantial interspecies (50%-60%) overall sequence homology with interspecies homologies increasing to about 56% to 99% in the cytoplasmic region of the protein, suggesting that they constitute a gene family (Nose, A., 1987; Miysysni, D., et al., 1989); and (6) a common mechanism of action, namely, homophilic binding of cadherins on one cell to similar cadherins on the adjoining cell.

CAMs independent of Ca^{2+} are also known, for example, the 125K glycoprotein of Urushihara et al. (Urushihara, H., et al., Cell, 20:363 (1980)); N-CAM (Rutishauser, U., Nature, Lond., 310:549 (1984)); Ng-CAM (Grunet, M. et al., Proc. Nat'l. Acad. Sci. (USA), 81:7989 (1984)); L1 (Rathjien, F.G. et al., J. Cell Biol., 3:1 (1984)); G4 (Rathjien, F.G. et al., J. Cell Biol., 104:343 (1987)); and platelet glycoprotein PECAM-1 (CD 31) (Newman, P.J., Science, 247:1219 (1990)). Ca^{2+} -independent CAMs are known to exhibit certain properties of the Ca^{2+} -dependent CAMs. Thus, N-CAM and N-cadherin both promote retinal neurite outgrowth on astrocytes (Neugebauer, K.M., et al., J. Cell Biol., 107:1177 (1985)), and on Schwann cells (Bixby, J.L. et al., J. Cell Biol., 107:353 (1988)).

Monoclonal antibodies raised against epithelial E-type cadherins such as uvomorulin are known to disrupt the adhesion of several cell types, including embryo cells, cultured teratocarcinoma cells, hepatocytes, and MDCK kidney epithelial cells (Ogou, S.-I., et al., J. Cell Biol., 97:944 (1983); Yoshida-Noro, et al., (1984), above; Shirayoshi, Y., et al., Cell Struct. Funct., 11:285 (1986); Gallin, et al., (1983), above; Vestweber, D., et al., EMBO J., 4:3393 (1985); Johnson, M.H., et al., J. Embrol. Exp. Morphol., 93:239 (1986); Gumbiner, B., et al., J. Cell Biol., 102:457 (1986)).

However, prior to the present discoveries disclosed in the parent applications cadherins had not been found in brain capillary or other endothelial cells (see, Takeichi, et al. (1988), above). Further, the CAMs of microvascular endothelial cells had not yet been identified, nor had such molecules been localized specifically to brain capillary endothelial cells. Thus, until the present invention no means were known for transiently and reversibly disrupting tight junctions between microvascular endothelial cells, including those of the BBB, based upon an attack upon the CAM's of such cells that are responsible for tight junction formation and maintenance.

It has been hypothesized that the cadherins contain a common cell adhesion recognition (CAR) sequence. The CAR sequences of several cell and substratum adhesion molecules are known. Martin, G.R., et al., Ann. Rev. Cell Biol., 3:57 (1987) ; Ruoslahti, E., et al., Science, 238:491 (1987). In general, CAR sequences are composed of at least three amino acid residues. The most rigorously investigated CAR sequence is RGD which is found in laminin, fibronectin and other basement membrane components that are responsible for the binding of cells to the substratum.

Blaschuk, et al., in a paper to be published subsequent to the filing of the present application (Blaschuk, O., et al., J. Mol. Biol., in press, (1990)), disclose the presence of three potential cadherin CAR sequences in the first extracellular domains of liver CAM, E-, P-, and N-cadherin, namely, PPI, GAD and HAV. Blaschuk, et al. (Blaschuk, O., et al., Develop. Biol., 139:227 (1990)), also disclosed recently that synthetic peptides containing the HAV sequence inhibited two biological processes (compaction of 8-cell-stage mouse embryos and rate of neurite

outgrowth on astrocytes) that are known to be mediated by cadherins. Effective peptides in these assays were LRAHAVDVNG and AHAVSE; PPI-containing peptides were without effect. However, Blaschuk et al. provide no
5 guidance for determining the regions flanking the HAV tripeptide that are critical for cell-cell adhesion. In the BBB disrupting peptides of the present invention detailed below, we have observed that the mere presence of the HAV sequence in a small cadherin-derived peptide
10 is not the sine qua non for a composition effective to prevent cell-cell adhesion. Indeed, it should be emphasized that neither Blaschuk et al. nor any other publication known to the present inventors suggest that cadherin sequences containing HAV or SHAVS sequences
15 would be effective in opening tight junctions and piercing blood brain barriers formed by E-cadherins in brain microvascular endothelial cells.

SUMMARY OF THE INVENTION

It has now been discovered that molecules
20 homologous to, and immunologically related to, cadherin cell adhesion molecules are present on brain and non-brain microvascular endothelial cells, such that

junctions between such endothelial cells can be reversibly opened so as to permit passage of therapeutic drugs by the use of polypeptide and antibody compositions that compete with such cell
5 adhesion molecules for binding to such cells.

It is therefore an object of this invention to provide the identity of microvascular endothelial cell adhesion molecules.

Another object of this invention is to provide DNA
10 sequences of genes, and plasmids containing same, coding for the expression of all or a cell-binding portion of microvascular endothelial cell adhesion molecules.

Yet another object of this invention is to provide
15 means to identify those sequences of cell adhesion molecules responsible for the tight binding of adjoining endothelial cells.

A further object is to provide therapeutic compositions comprising polypeptides derived from cell
20 adhesion molecules that reversibly disrupt cell-cell adhesion.

Still another object of this invention is to provide therapeutic compositions comprising polyclonal or monoclonal antibodies or fragments thereof directed
25 against endothelial cell adhesion molecules, or against polypeptides representing cell binding regions thereof, that reversibly disrupt endothelial cell-cell adhesion.

Yet another object of this invention is to provide therapeutic formulations comprising therapeutic drugs
30 conjugated with blood-brain barrier-disrupting compositions of this invention, that are capable of entering the central nervous system following disruption of the blood-brain barrier.

These and other objects of this invention will
35 become clear by reference to the following description

of the invention and to the appended claims.

DESCRIPTION OF THE DRAWINGS

Figure 1 illustrates the partial cDNA sequence for bovine endothelial cell adhesion molecule homologous to chicken N-cadherin.

Figure 2 illustrates the partial cDNA sequence for bovine endothelial cell adhesion molecule homologous to mouse P-cadherin.

Figure 3 illustrates the cDNA sequence for the MDCK cell adhesion molecule homologous to mouse E-cadherin.

Figure 4 illustrates the restriction sites in the bovine endothelial cell N- (4-1 to 4-5) and P-cadherin (4-6 to 4-8) cDNA sequences and in the MDCK E-cadherin (4-9 to 4-14) cDNA sequence.

Figure 5 shows the staining of a mouse brain thin section by an antibody raised against a fusion protein derived from amino acids 9-96 of MDCK E-cadherin containing an HAV region.

Figure 6 is a repeat of the experiment of Fig. 5, except that the antibody was raised against the entire E-cadherin molecule.

Figure 7 illustrates the effects of an 18-mer HAV-containing polypeptide on the resistance of tight junction monolayers of MDCK epithelial cells.

Figure 8 illustrates the effects of 11-mer and 18-mer HAV-containing polypeptides on the resistance of tight junction monolayers MDCK epithelial cells.

Figure 9 illustrates the effects of 11-mer and 18-mer HAV-containing polypeptides on the resistance of tight-junction monolayers of brain microvascular endothelial cells.

DETAILED DESCRIPTION OF THE INVENTION

It has now been discovered that cell adhesion molecules with characteristics of cadherins are present on the surfaces of brain capillary endothelial cells and of microvascular endothelial cells of non-brain origins. The present invention is based on the discovery that a polypeptide composition comprising cell binding domains of endothelial cell adhesion molecules may compete against such molecules for binding to such cells, such that by this means the junctions between such cells could be reversibly opened, thereby permitting penetration by therapeutic agents. The present invention also discloses that polyclonal or monoclonal antibodies (or fragments thereof) raised against endothelial cell adhesion molecules or cell-binding domains thereof may also compete for endothelial cell surface binding sites, and, by this means, reversibly disrupt junctions between endothelial cells, thereby permitting entry into the central nervous system of therapeutic agents.

In order to obtain compositions useful for disrupting tight junctions between microvascular endothelial cells, the cell adhesion molecules responsible for such junctions were identified.

The endothelial cell cadherins disclosed herein exhibit one or more of several characteristics of E-, P- and N- cadherins, including: characteristics of a transmembrane integral protein, with cytoplasmic, hydrophobic plasma membrane, and extracellular regions; intraspecies DNA sequence homologies of greater than about 50% for the entire molecule; immunological cross-reactivity with antibodies raised against non-endothelial cell cadherins; and containing cell-binding domains. "Immunologically related to" means that these cadherin-like molecules cross-react with antibodies

raised against non-endothelial cell cadherins.

E-cadherin-like molecules were localized in brain by immunofluorescence. Cryostat sections of mouse brain were labeled with a rabbit antibody prepared
5 against E-cadherin, and then with fluorescein isothiocyanate-conjugated goat anti-rabbit immunoglobulin. There is clear labeling of a capillary in brain sections as shown by immunofluorescence microscopy. Endothelial cells in liver and kidney were
10 not stained by this procedure.

cDNAs coding for the expression of bovine microvascular endothelial cell (BMEC) cadherins were cloned and sequenced as described below, and the partial sequence of N-cadherin and P-cadherin are
15 disclosed herein in Figures 1 and 2, respectively. In addition, as MDCK dog kidney epithelial cells are known to employ E-cadherin to form high resistance tight junctions, and as the present invention discloses that brain capillary endothelial cell adhesion molecules
20 include E-type cadherin, the DNA of this cadherin was also cloned; its complete DNA sequence is disclosed herein (Fig. 3).

N-, P- and E-cadherin-type clones described herein were deposited in the American Type Culture Collection
25 on September 26, 1989, and were assigned the following accession numbers:

	<u>Clone Designation</u>	<u>Accession No.</u>
	N-cadherin-type clones	
	pUC19-bNCad 10A	40667
	pUC19-bNCad 39A	40669
5	P-cadherin-type clones	
	pUC18-bPCad 3B-10	40668
	pUC19-bPCad 9B	40670
	E-cadherin-type clones	
	pBluescript MDCKECad 45-30E	40671

10 The cloning of cadherins was accomplished by taking advantage of the fact that the cadherins characterized thus far are transmembrane glycoproteins, the cytoplasmic domains of which are highly conserved, that is, are highly homologous.

15 Two degenerate oligonucleotides flanking the 42-amino acid coding region in the cytoplasmic domain were selected to serve as primers for polymerase chain reaction (PCR) using either BMEC cDNA or MDCK cDNA as templates. The PCR reactions were carried out
20 essentially according to Saiki, R. K. et al., Science, 239:487 (1988), which is incorporated herein by reference.

The cloned PCR products from each cell type were sequenced essentially according to the method of
25 Sanger, F. et al., Proc. Nat'l. Acad. Sci. (USA), 74:5463 (1977), which is incorporated herein by reference.

It was discovered that BMEC cadherins are of two types - one homologous to chicken N-cadherin (neuronal
30 type, see, e.g., Hatta, K., et al., J. Cell Biol., 106:873 (1988)) and the other homologous to mouse P-cadherin (placental type, see e.g., Nose, A., et al., (1987) above). It has also been found that there are two species of cadherins in MDCK cells - one homologous

to mouse E-cadherin (see, e.g., Nagafuchi, A., et al., Nature, 329:341 (1987)) and the other homologous to mouse P-cadherin (Nose, et al. (1987), above).

The PCR products were then used as probes to
5 isolate the BMEC and MDCK cadherin cDNA clones as follows. A cDNA library was constructed essentially according to Gubler et al. (Gubler, U. et al., Gene, 25:263 (1983), which is incorporated herein by reference), using poly (A)⁺RNA isolated from either
10 BMEC or MDCK cells. The cDNA was ligated via EcoRI adaptors into gt10 arms (BMEC) or ZAP^R (from Stratagene, Inc., La Jolla, CA) vector arms (MDCK). cDNA libraries containing 5×10^5 - 1.5×10^6 independent cDNA clones were screened using
15 radiolabeled PCR products (Benton, W.D. et al., Science, 196:180 (1987), which is incorporated herein by reference). Northern blot analysis (Maniatis, T. et al., "Molecular Cloning: A Laboratory Manual", Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.,
20 1982) may be used to determine whether each cDNA species cloned hybridizes to a single mRNA species, as well as the tissue distributions of each cDNA species.

cDNA clones for each cadherin were sequenced by the method of Sanger et al. (1977) above.

25 The partial restriction maps for each cDNA clone based on their sequences are shown in Fig. 4. Some of these restriction sites were confirmed by restriction enzyme digestions, including Hind III, Pst I, Kpn I, Bgl II for N-cadherin; Pvu II, Sac I and Pst I for
30 P-cadherin; Pst I, Pvu II, BamH I, and Sac I for E-cadherin.

In order to test whether the cloned E-cadherin cDNA contains all the information necessary for cadherin function, full-length E-cadherin cDNA joined
35 to a suitable promoter may be introduced into mouse

L-cells that have very little endogenous cadherin activity (Nagafuchi, et al. (1987), supra). To test for expression of E-cadherin in transfectants derived from the introduced cDNA, transfected L-cells may be tested for Ca^{2+} -dependent aggregating activity. The extent of this aggregating activity should be closely correlated with the amount of E-cadherin expressed (Takeichi, M. (1988), supra). This same technique may be used for testing cDNAs encoding bovine endothelial N- and P-cadherins, according to the method of Hatta, et al. (Hatta, K., et al. (1988), supra).

In order to identify cell binding domains in, for example, MDCK E-type cadherin, L-cells may be first transfected as above with a cDNA of a size sufficient to cause Ca^{2+} -mediated aggregation of transfectants. A series of deletion mutants comprising truncated cDNA species missing different regions of the extracellular domain may be prepared by restriction enzyme digestion and proper end filling or exonuclease digestion to make the deletions in the proper coding frames. These deletion mutants can then be tested for their ability to express in L-cells a protein causing Ca^{2+} -dependent aggregation. By correlating a loss of aggregation with deletion of particular fragments, the regions important for cell binding may be determined. A variety of polypeptides corresponding to binding regions of cadherins, as deduced from the nucleotide sequences of deleted cDNA, may be synthesized chemically using an automated peptide synthesizer such as that of Applied Biosystems, Inc., Foster City, CA, or expressed by recombinant DNA methods. Effective polypeptides may be of varying lengths, depending upon the natures of junctions being disrupted and the cell adhesion molecule present.

Nucleotide, and corresponding amino acid, sequences of cadherins may be analyzed to detect homologous regions. Applying this technique to bovine endothelial cell N- and P-cadherins and to epithelial cell E-cadherin, we have determined that, in the amino acid 80 region of each of these cadherins, there is conserved a triplet HAV (His-Ala-Val) region. We have deduced that this HAV region may be a common cell adhesions recognition (CAR) sequence.

We have chemically synthesized the following polypeptides, each of which containing the HAV sequence:

6-mer(78-83)	NH ₂ -SHAVSS-CONH ₂
11-mer(76-86)	NH ₂ -LYSHAVSSNGN-CONH ₂
17-mer(74-90)	NH ₂ -YILYSHAVSSNGNAVED-CONH ₂
18 mer(69-86)	NH ₂ -EQIAKYILYSHAVSSNGN-CONH ₂
20-mer(71-90)	NH ₂ -IAKYILYSHAVSSNGNAVED-CONH ₂

and have tested each for efficacy in opening brain endothelial cell tight junctions in the BBB model disclosed in copending United States application Serial No. 07/413,274, and also on kidney epithelial cell tight junctions..

Polyclonal antibodies raised in rabbits and monoclonal antibodies derived from hybridomas may be generated against each of the chemically-synthesized polypeptides by standard methods. (Harlow, E., et al., "Antibodies: A Laboratory Manual", Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988; Goding, J.W., "Monoclonal Antibodies: Principles and Practice", Academic Press, N.Y. 1986). In addition, recombinant antibodies may be prepared. Fragments of antibodies, e.g., Fc, Fab, F(ab)', may be prepared by standard methods.

We have cloned and sequenced fusion proteins derived from amino acids 9-96 of MDCK E-cadherin

containing the HAV region. A polyclonal antibody prepared against this fusion protein stained rat (Fig.55) mouse brain sections as well as did an antibody raised against the entire E-cadherin (Fig. 6).

5 A polyclonal antibody raised against a fusion protein derived from amino acids 9-37 failed to stain brain sections. These results indicate that the key cell-binding domain of E-cadherin lies in the region of amino acids 37-96.

10 The ability of CAM-derived polypeptides containing cell-binding domains, and the corresponding polyclonal and monoclonal antibodies, of the invention to disrupt tight junctions may be tested in in vitro and in vivo models of high resistance tight junctions and in animal
15 models. Monolayers of MDCK dog kidney epithelial cells, that are known to contain high resistance tight junctions (Gumbiner, B., J. Cell Biol., 102:457 (1986)), can be used to test for the ability of the polypeptides and corresponding antibodies of the
20 present invention to disrupt such tight junctions.

Polyclonal antibodies prepared as described above may also be used in conjunction with Western blotting (Old, R.W., et al., Principles of Gene Manipulation, 3d ed., Blackwell, Oxford, 1985, p. 10) and a variety of
25 tissue extracts in order to identify cell adhesion glycoproteins in such extracts.

Another embodiment of the present invention is in drug delivery systems. Conjugates between therapeutic drugs and agents that affect cell adhesion molecule
30 function in brain capillary endothelial cells may be used to deliver therapeutic drugs to the CNS. For example, a polypeptide derived from a cell adhesion molecule that contains within its amino acid sequence a cell-binding domain, or antibodies thereto, may be
35 conjugated in biologically-active form to a therapeutic

modality. Such conjugates may have the dual effect of opening the BBB and delivering the therapeutic agent to the brain side of the BBB. Delivery of therapeutic drugs to the CNS, either alone or conjugated to agents that disrupt cell-cell adhesion, may be accomplished by administering such drugs to a subject either simultaneously with or subsequent to the administration of the agents of this invention that disrupt the tight junctions of the BBB. Examples of therapeutic modalities that may be delivered to the brain by the cell adhesion disruption compositions of this invention include Nerve Growth Factor, anti-Parkinsonian drugs, and brain enzymes known to be missing in sphingolipidoses, e.g., Tay-Sachs disease. Means of chemically conjugating protein or polypeptide carriers to therapeutic agents such that the biological integrity of the therapeutic agent is not compromised and such that the therapeutic agent is readily cleaved from the carrier by enzymes present on or within endothelial cells (e.g., amidases, esterases, disulfide-cleaving enzymes), are well known in the art. It is also apparent that these therapeutic conjugates may be delivered to endothelial cells in encapsulated form (e.g., in liposomes) or as microsuspensions stabilized by pharmacological excipients.

It is known (Jain, R.K., J. Natn'l Cancer Inst., 81:570 (1989)) that many solid tumors develop internal barriers, including high pressure zones and collapsed blood vessels, that make it difficult for blood-borne chemotherapeutic agents to reach the tumor's inner core. The barrier problem is particularly troublesome with therapeutic products drawn from the human immune system, such as monoclonal antibodies conjugated with chemotherapeutic agents, interleukin-2, interferon and activated killer T-lymphocytes, because of their large

size. Thus, in another embodiment of this invention, compositions that disrupt the junctions between endothelial cells, particularly the relatively small peptides that contain one or more cell-binding regions of cell adhesion macromolecules, may be used to enhance drug delivery to tumors with depressed blood flow.

It has been theorized that cancer cells metastasize by secreting soluble cadherins variously to open tight junctions in cells that block their movement and to prevent their being bound to such cells. We consider it likely that antibodies raised against these cadherins, which are derived from extracellular domains of the cadherins disclosed in this invention, may provide a therapeutic modality that inhibits or prevents cancer cell metastases.

In another embodiment, the compositions of this invention may also be used to provide penetration for chemotherapeutic agents of other well-known blood-tissue barriers, such as blood-testis barriers and blood-retina barriers. The latter barrier is known to prevent the efficient transport of, for example, administered antibiotics to the retina from the general circulation. The cell adhesion disrupting compositions of this invention may, thus, be used in conjunction with the administration of antibiotics to treat retinal infections.

The following examples are illustrative of several embodiments of this invention, and should not be construed in any way as limiting the invention as recited in the claims.

EXAMPLE 1

EFFECTS OF HAV-CONTAINING POLYPEPTIDES ON TIGHT JUNCTIONS OF MDCK EPITHELIAL AND BOVINE ENDOTHELIAL CELLS

The BBB model of copending U.S. Serial No. 07/413,332 was used to examine the effects of polypeptides containing the HAV region on the tight junctions of monolayers of MDCK epithelial cells and
 5 bovine capillary endothelial cells as determined by resistance measurements across the monolayers.

The polypeptide was added to the cells either from the apical side (top) or basolateral side (bottom), as shown in the following sketch.

10

APICAL

EPITHELIAL CELLS
 Gut Side

ENDOTHELIAL CELLS
 Blood Side

Blood Side

Brain Side

BASOLATERAL

15

Figure 7 illustrates the effects of various concentrations of the aforementioned 18-mer polypeptide on resistance of MDCK epithelial cells. At the lowest concentration tested, 0.5 mg/ml, resistance was markedly decreased. The polypeptide was more effective
 20 when added from the basolateral side, but at high concentrations was quite effective even when added from the apical side. These data indicate that the 18-mer is effective in making tight junctions permeable. The 20-mer was similarly effective, and a 17-mer less
 25 effective.

30

Figure 8 illustrates the effects of the aforementioned 11-mer and 18-mer on MDCK cell resistance when added from either the apical or basolateral side of the monolayers. The concentration
 of polypeptide was about 1 mg/ml. The 11-mer (as well

as the 6-mer data not shown) was virtually without effect. With the 18-mer, resistance was almost totally abolished by about 6 hours, indicating disruption of tight junctions. That the effect of the 18-mer is reversible is indicated by the "wash-out" experiment. When the 18-mer was washed out of the MDCK cells at 6 hours, resistance recovered to a substantial extent over the next 21 hours. This recovery was particularly pronounced when the 18-mer had originally been added from the basolateral side of the monolayers. The 20-mer produced results similar to those of the 18-mer, and the 17-mer was effective, but somewhat less so.

Figure 9 illustrates the effect of 1 mg/ml of the 11-mer and 18-mer on high resistance monolayer cultures of brain endothelial cells (see copending United States Serial No. 07/413,332 for method of preparation). As with MDCK cells, the 11-mer (and the 6-mer) failed to reduce resistance values over a 48-hour period of observation. In contrast, the 18-mer (as well as the 20-mer) decreased resistance values markedly when added from either the basolateral or apical side, but the effect of the polypeptide was more rapid and more pronounced when it was added from the basolateral side; the 17-mer was less effective.

The conclusion of these experiments is that a particular set of peptides (but not all peptides) centered around the HAV region of E-cadherin are effective in opening tight junctions of brain endothelial cell blood-brain barriers, and also of epithelial cells that form such junctions ("gut barrier"). Both the length and composition of the amino acid region flanking the HAV triplet thus appear to play a role in the efficacy of such compositions.

While the aforementioned embodiments represent the preferred embodiments of the invention, those skilled

in the art may, without undue experimentation, devise other executions of the compositions and methods of use of this invention without departing from the concept and spirit inherent therein.

What is claimed is:

1. A composition for opening tight junctions between microvascular endothelial cells of a subject, whereby means are provided for a drug to cross the permeability barrier imposed by such junctions,
5 comprising an agent capable of reacting with at least one type of cell-bound cell adhesion molecule that would otherwise mediate tight junction formation between microvascular endothelial cells, so that cell-cell adhesion is disrupted.
2. A composition of claim 1, wherein said cell adhesion molecule exhibits at least about 50% sequence homology with a cadherin selected from the group consisting of E-cadherin, N-cadherin and P-cadherin.
3. A composition of claim 1, wherein said cell adhesion molecule is immunologically related to at least one of the group consisting of E-cadherin, N-cadherin and P-cadherin.
4. A composition of claim 1, wherein the microvascular endothelial cells are brain capillary endothelial cells.
5. A composition of claim 2, wherein said agent comprises an inhibitor of the binding to cells of said cell adhesion molecule.
6. A composition of claim 3, wherein said agent comprises an inhibitor of the binding to cells of said cell adhesion molecule.
7. A composition of claim 5, wherein said inhibitor agent comprises a fragment of said cell adhesion molecule.
8. A composition of claim 7, wherein said cell adhesion molecule fragment includes within its amino acid sequence a cell-binding domain.

9. A composition of claim 8, wherein said cell-binding domain contains an HAV amino acid sequence.

10. A composition of claim 9, wherein said amino acid sequence is



11. A composition of claim 9, wherein said amino acid sequence is



12. A composition of claim 9, wherein said amino acid sequence is



13. A composition of claim 9, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

14. A composition of claim 5, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against said cell adhesion molecule.

15. A composition of claim 5, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against a fragment of said cell adhesion molecule.

16. A composition of claim 15, wherein said cell adhesion molecule fragment includes within its amino acid sequence a cell-binding domain.

17. A composition of claim 16, wherein said cell-binding domain contains an HAV amino acid sequence.

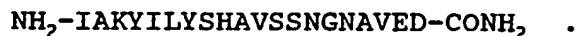
18. A composition of claim 17, wherein said amino acid sequence is



19. A composition of claim 17, wherein said amino acid sequence is



20. A composition of claim 17, wherein said amino acid sequence is



21. A composition of claim 17, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

22. A composition of claim 5 or 6 in a pharmaceutically-acceptable vehicle.

23. A method for opening tight junctions between microvascular endothelial cells of a subject, comprising the step of administering to the subject an agent, in an effective amount and in a
5 pharmaceutically-acceptable vehicle, capable of reacting with at least one type of cell-bound cell adhesion molecule that would otherwise mediate tight junction formation between microvascular endothelial cells, so that cell-cell adhesion is disrupted and
10 whereby means are provided for a drug to cross permeability barriers imposed by such tight junctions.

24. A method of claim 23, wherein said cell adhesion molecule exhibits at least about 50% homology with a cadherin selected from the group consisting of E-cadherin, N-cadherin and P-cadherin.

26

25. A method of claim 23, wherein said cell adhesion molecule is immunologically related to at least one of the group consisting of E-cadherin, N-cadherin and P-cadherin.

26. A method of claim 23, wherein the microvascular endothelial cells are brain capillary endothelial cells.

27. A method of anyone of claims 23-25, inclusive, wherein said agent comprises an inhibitor of the binding to cells of said cell adhesion molecule.

28. A method of claim 27, wherein said inhibitor agent comprises a fragment of said cell adhesion molecule.

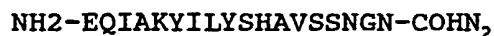
29. A method of claim 28, wherein said cell adhesion molecule fragment includes within its amino acid sequence a cell-binding domain.

30. A method of claim 29, wherein said cell-binding domain contains an HAV amino acid sequence.

31. A method of claim 30 wherein said amino acid sequence is



32. A method of claim 30, wherein said amino acid sequence is



33. A method of claim 30, wherein said amino acid sequence is



34. A method of claim 30, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

35. A method of claim 27, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against said cell adhesion molecule.

36. A method of claim 28, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against said fragment of said cell adhesion molecule.

37. A method of claim 36, wherein said cell adhesion fragment includes within its amino acid sequence a cell-binding domain.

38. A method of claim 37 wherein said cell-binding domain contains an HAV amino acid sequence.

39. A method of claim 38, wherein said amino acid sequence is



40. A method of claim 38, wherein said amino acid sequence is



41. A method of claim 38, wherein said amino acid sequence is



42. A method of claim 38, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

43. A drug delivery composition comprising a conjugate between a therapeutic drug and an agent capable of reacting with at least one type of a cell-bound cell adhesion molecule that would otherwise
5 mediate tight junction formation between microvascular endothelial cells, so that cell-cell adhesion is

disrupted by said agent, whereby means are provided for said drug to cross permeability barriers imposed by such tight junctions, in a pharmaceutically-acceptable
10 vehicle.

44. A drug delivery composition of claim 43, wherein said cell adhesion molecule exhibits at least about 50% homology with a cadherin selected from the group consisting of E-cadherin, N-cadherin and P-cadherin.

45. A drug delivery composition of claim 43, wherein said cell adhesion molecule is immunologically related to at least one of the group consisting of E-cadherin, N-cadherin and P-cadherin.

46. A drug delivery composition of claim 43, wherein the microvascular endothelial cells are brain capillary endothelial cells.

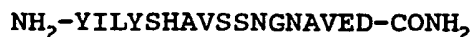
47. A drug delivery composition of any one of claims 43-45, inclusive, wherein said agent comprises an inhibitor of the binding to cells of said cell adhesion molecule.

48. A drug delivery composition of claim 47, wherein said agent comprises a fragment of said cell adhesion molecule.

49. A drug delivery composition of claim 48, wherein said cell adhesion molecule fragment includes within its amino acid sequence a cell-binding domain.

50. A drug delivery composition of claim 49, wherein said cell-binding domain contains an HAV amino acid sequence.

51. A drug delivery composition of claim 50, wherein said amino acid sequence is



52. A drug delivery composition of claim 50, wherein said amino acid sequence is



53. A drug delivery composition of claim 50, wherein said amino acid sequence is



54. A drug delivery composition of claim 50, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

55. A drug delivery composition of claim 43, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against said cell adhesion molecule.

56. A drug delivery composition of claim 43, wherein said inhibitor agent comprises a polyclonal or monoclonal antibody directed against a fragment of said cell adhesion molecule.

57. A drug delivery composition of claim 56, wherein said cell adhesion molecule fragment contains within its amino acid sequence a cell-binding domain.

58. A drug delivery composition of claim 56, wherein said cell-binding domain encompasses an HAV amino acid sequence.

59. A drug delivery composition of claim 58, wherein said amino acid sequence is



60. A drug delivery composition of claim 58, wherein said amino acid sequence is

NH₂-EQIAKYILYSHAVSSNGN-COHN₂ .

61. A drug delivery composition of claim 58, wherein said amino acid sequence is

NH₂-IAKYILYSHAVSSNGNAVED-CONH₂ .

62. A drug delivery composition of claim 58, wherein said amino acid sequence comprises amino acids 9-96 of E-cadherin.

63. A drug delivery composition of claim 43, wherein said conjugate comprises a physiologically-cleavable covalent bond.

64. A drug delivery composition of claim 43, wherein said conjugate is encapsulated within a physiologically-compatible particle.

65. A drug delivery composition of claim 64, wherein said particle comprises a liposome.

FIG. 1a.

Partial cDNA sequence for the bovine endothelial N-cadherin

GAATTCGAAC	CCCTTCGTTT	CATTATGCAA	GACTGGATTT	CCTGAAGATG	TGTACAGTGC	60
AGTCTTGTC	CGGGATGTGC	TGGAAGGACA	GCCCTTCTC	AATGTGAAGT	TTAGCAACTG	120
CAATGGGAA	AGAAAAGTAC	AGTATGAGAG	CAGCGAGCCA	GCAGATTTTA	AGGTGGATGA	180
AGATGGCATG	GTGTATGCCG	TGAGAAGCTT	CCCCCTCTCA	TCTGAACACT	CGAAGTTCCT	240
GATATACGCT	CAAGACAAAG	AGACTCAGGA	AAAGTGGCAA	GTAGCAGTAA	AACTGAGCCT	300
CAAACCAAGC	CTACCTGAGG	ATTCAGTGAA	GGAATCACGA	GAAATAGAAG	AAATAGTGTT	360
TCCAAGACAA	GTGACTAAGC	ACAATGGCTA	CCTGCAGAGG	CAGAAGAGAG	ACTGGGTTAT	420
CCCTCCCATC	AACTTGCCAG	AAACTCCAG	AGGGCCTTTT	CCTCAAGAGC	TCGTCAGGAT	480
CAGATCCGAT	AGAGATAAAA	ACCTTTCTCT	GCGGTACAGC	GTAAC TGGGC	CAGGAGCTGA	540
CCAGCCTCCA	ACTGGTATCT	TCATTATCAA	CCCCATCTCA	GGTCAGCTGT	CAGTAACCAA	600
GCCTCTGGAT	CGTGAGCTGA	TAGCCCGGTT	TCATTTGAGG	GCACATGCAG	TGGATATTAA	660
TGGAACACAA	GTGGAGAACC	CCATCGACAT	TGTCATCAAC	GTTATTGACA	TGAATGATAA	720
CAGACCTGAG	TTCTTACACC	AGGTTTGGAA	TGGGACAGTT	CCTGAGGGAT	CAAAGCCGGG	780
AACATATGTG	ATGACGGTCA	CTGCGATTGA	TGCTGACGAT	CCAATGCCC	TCAATGGGAT	840

1/42

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FIG. 1b.

GTTGAGGTAC AGAATCCTGT CCCAGGGCGCC AAGCACCCCT TCGCCCAACA TGTTTACAAT	900
CAACAATGAG ACTGGGGACA TTATCACGGT GGCAGCTGGA CTTGACAGAG AAAAAGTACA	960
ACAGTATACG TTAATAATTTC AAGCTACAGA CATGGAAGGC AATCCACAT ATGGCCTTTC	1020
CAACACAGCC ACGGCTGTCA TCACGGTGAC AGATGTCAAC GACAATCCTC CGGAGTTTAC	1080
TGCCATGACG TTCATATGGTG AAGTCCCTGA AAACAGGGTA GATGTCAATCG TCGCTAATCT	1140
AACAGTGACA GATAAGGATC AGCCCCACAC ACCGGCCTGG AACGCCATCT ACAGAAATCAG	1200
CGGTGGAGAC CCCGCCGGCC GCTTTGCCAT TCAAACTGAC CCCAACAGCA ACGACGGTTT	1260
AGTCACCGTA GTAAAACCAA TCGACTTTGA AACAAATAGG ATGTATGTCC TTACTGTCCG	1320
TGCAGAAAAT CAAGTGCCAT TAGCCAAGGG TATTCAGCAT CCACCTCAGT CAACTGCGAC	1380
TGTGTCTGTC ACAGTTATCG ATGTGAATGA AAATCCTTAT TTTGGCCCCAA ATCCAAAAGAT	1440
CATTGCGCAA GAAGAAGGCC TTACAGCCCGG TACCGTGTTA ACAACGTTTA CTGCTCAGGA	1500
CCCAGATCGA TATATGCAGC AAAATATCAG ATACACCAA TTATCCGATC CTGCAAACTG	1560
GCTAAAAATA GACTCTGTGA ATGGGCAGAT AACTACCAT GCTGTTTTGG ACAGAGAAATC	1620
ACCGAATGTG AAAGCCAATA TATACAATGC TACTTTCCCTT GCTTCTGACA ATGGAATCCC	1680
TCCTATGAGT GGAACGGGAA CACTGCAGAT CTATTACTT GATATTAATG ACAATGCCCC	1740

2/42

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FIG. 1c.

TCAAGTGTTA	CCTCAAGAGG	CAGAGATTTG	TGAAACTCCG	GACCCCAATT	CAATTAAACAT	1800
CACAGCACTT	GATTATGACA	TTGATCCAAA	TGCTGGACCA	TTTGCTTTTG	ATCTTCCCTTT	1860
GTCTCCAGTG	ACTATTAAAG	GAAATTGGAC	CATCACTCGG	CTTAATGGTG	ATTTTGCTCA	1920
GCTTAACTTA	AAGATAAAAT	TTCTTGAGGC	CGGATCTAC	GAAGTTCCAA	TCATAATCAC	1980
AGATTGGGGT	AATCCTCCCA	AATCGAATAT	CTCCATCCTT	CGGGTGAAGG	TTTGCCAGTG	2040
TGATTCCCAAC	GGGACTGCA	CAGATGTGGA	TCGAATTGTG	GGAGCAGGGC	TGGGCACCGG	2100
CGCCATCATC	GCCATCCTGC	TTTGCAATCAT	CATCCTGCTC	ATTCTCGTTC	TGATGTTTCGT	2160
GGTATGGATG	AAACGCCGGG	ATAAGAAGCG	CCAGGCCAAA	CAACTTTTAA	TTGATCCAGA	2220
AGATGATGTA	AGAGATAATA	TTTTAAATA	TGATGAAGAA	GGTGGAGGAG	AAGAAGACCA	2280
GGACTACGAT	TTGAGCCAGC	TCCAGCAGCC	TGATACGGTA	GAGCCAGATG	CCATCAAGCC	2340
AGTTGGAATC	CGACGGTTGG	ATGAGAGGCC	CATCCATGCG	GAGCCCCAGT	ACCCGGTTCG	2400
ATCTGCAGCC	CCACACCCAG	GGGACATCGG	GGACTTCATT	AATGAGGGCC	TTAAAGCTGC	2460
TGACAAACGAT	CCCACCGCTC	CGCCCTACGA	CTCCCTCTTA	GTCTTTGACT	ATGAAGGCAG	2520
TGGCTCCACG	GCCGGGTCCT	TGAGCTCCCT	TAATTCCCTCC	AGTAGTGGAG	GTGAGCAGGA	2580
CTATGACTAT	CTGAACGACT	GGGGGCCCCC	CTTCAAGAAA	CTCGCTGACA	TGTACGGTGG	2640

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FIG. Id.

AGGTGATGAC TGAACCTCAG GGTGAACTTG GTTTTGGAC AAGTACAAAC AATTGCAACT 2700
GATATTCCCA AAAAGCATTC AGAAGCTAGG CTTTAACTTT GTAGTCTACT AGCACAGTGC 2760
TTGCTGGAGG CTTTGGCAGA GGCTGCAAAC CAATTGGGC TCAGAGGGAA TATCGGTGAT 2820
CCAATACTGT TTGGAAAACA CTGAGCTCAG TTACACTTGA ATTTTACAGT ACAGAAAGCAC 2880
TGGGATTTTA TGTGCCCTTTT TGTACCTTTT TCAGATTGGA ATTAGTTTTA TGTTTAAGGC 2940
TTTAATGGTA CTGATTTC TG AAATGATAAG TAAAAGACAA AATATTTTGT GGTGGGAGCA 3000
GTAAGTTAAA CCATGATATG CTTTCGACACG CTTTGTGTAC ATCGCATTTG CTTTATTATA 3060
AAATATGGAA TTAAACAGAC AAACCAACCA CTCATGGAGC AATTTTATTA CCTTGGGGC 3120
TGAGACCATG AGATTGGAAA ATGTACATTA TTCTCTAGTTT TAGACTTTAG TTTCTTGTTT 3180
TGTTTTTTT TTCCACTAAA ATCTTAAAC TTACGCAGCT GGTGCAAAT AAAGGGAGTT 3240
TTCATATCAC CAATTGTAG CAAAATTGAA TTTTTCATA AACTAGAATG TTAGACACAT 3300
TTTGGTCTTA ATCCATGTAC ACTTTTTTAT TTA CTGTATT TTTTCCACTT CACTGTAAAA 3360
ATGGTATGT TACATAATGT TTTATTGGCA TAGTCTATGG AGAAGTGCAG AAACCTCAGA 3420
ACATGTGTAT GTATTATTG GACTATGGAT TCAGGTTTTT TGCATGTTTA TATCTTTCGT 3480
TATGGATAAA GTATTTACAA AACAAAGTGA CATTGATTC AATTGTTGAG CTGTAGTTAG 3540
AATACTCAAT TTTTAAATTTT TTAATTTTTT TTATTTTTTA TTTTCTCTTT TTGTTTGGG 3600

AGGGAGAAA GTTCTTAGCA CAAATGTTTT ACATAATTG TACCAAAAAA AAACAAAAA 3660
AAAGGAAAGA CAAGAAATGA AAGGGGTGAC CTGACACTGG TGGTACTACT GCAGTGTGTG 3720
TTTTTAAAAA AAAATGAAAA AAAAAAGCT TTAAACTGG AGAGACTTCT GACAACAGCT 3780
TTGCCCTCTGT ATTGTGTACC AGAATATAAA TGATACACCT CTGACCCAG CGTCTGAAT 3840
AAAATGCTAA TTTTGGAAAA AAAAAAAA AAAA 3875

FIG. 1e.

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FIG. 2a.

partial cDNA sequence for the bovine endothelial P-cadherin

GAATTGGAAC CCTTCGCTG AGAACACAGT GAGCCACGAG GTGCAGAGGC TGACAGTGAC 60
TGATCTGGAC GCCCCTAACT CACCAGCATG GCGTGCCACC TACCGCATCG TGGGAGGTGA 120
CAACGGGGAC CATTTTACCA TCACTACTGA CCCCAGAGAGC AACGAGGGTA TCCTGACCAC 180
CCAGAAGGGC TTGGATTTTG AGGCCAAAAC CCAGCACACC CTGTACGTGG AAGTGATCAA 240
CGAGGTCCC TTTGTGGTGA AACTCCCGAC CTCACAGCC ACCGTAGTGG TCCTCGTGGA 300
GGATGTGAAT GAGCCACCGG TGTTTGTCCC CCCGTCCAAA GTCATCGAAA TCCAGGAGGG 360
CATCTCCACT GGGGAGCCTA TTTGTGCCTA CACTGCCCGG GACCCAGACA AGGGGAGTCA 420

FIG. 2b.

6/42

GAAGATCAGT TACCACATCC TGAGAGACCC AGCAGGGTGG CTAGCGATGG ACCCAGACAG 480
TGGACAAGTC ACTGCCGCAG GGGTCTTGGA CCGTGAGGAT GAGCAGTTTG TGAGAAACAA 540
CATCTACGAA GTCATGGTCT TGGCCACAGA TGATGGGAGC CCTCCCACCA CTGGCACAGG 600
GACCCCTCCTG CTAACACTGA TGGACATCAA TGACCACGGT CCGGTCCCCG AGCCCCGTCA 660
GATCACCATC TGCAACCAAA GCCCTGTGCC CCAGGTGCTA AACATCACAG ACAAGGACTT 720
GTCCCCCCAC ACTGCCCCCTT TCCAGGCCCA ACTCACACAT GACTCGGACG TCTATTGGAC 780
AGCAGAAAGTC AACGAGAAAG GAGACGCAGT AGCCTTGTCC CTGAAGAAGT TCCTAAAGCA 840
AGGCCAATAC GATGTGCACC TTTCCCTGTC CGACCACGGC AACAAAGAAC AGCTGACAGT 900
GATCAGAGCC ACCGTGTGTG ACTGCCACGG CAACATGGTG ACCTGCCGGG ACCCCTGGAC 960
GTGGGGTTTC CTCCTCCCCA TCCTGGGTGC TGCCCTGGCT CTGCTGCTCC TTCTGCTGGT 1020
GCTCCTATTTC TTGGTGAGAA AGAAACGGAA GATCAAGGAA CCCCTTCTCC TCCCAGAAGA 1080
TGATAACCCGT GACAACGTCT TCTACTACGG CGAAGAGGGG GGTGGCGAGG AGGACCAGGA 1140
CTATGACATC ACCCAGCTCC ACCGGGGTCT GGAGGCCCGG CCTGAGGTGG TTCTCCGCAA 1200
CGATGTGGCA CCATCCTTCA TCCCCACACC CATGTACCGT CCTCGGCCAG CCAACCCAGA 1260
TGAAATCGGC AACTTCATCA TTGAGAACCT GAAGGCAGCC AACACAGACC CCACGGCCCC 1320

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GCCTACGAC TCCCTGTTGG TGTTGACTA TGAGGGCAGT GGCTCCGATG CCGCCTCTCT 1380
GAGCTCGCTC ACCTCCTCAA CCTCTGACCA GGACCAAGAC TACAACATATC TGAATGAGTG 1440
GGCAGCCGC TTCAAGAAGC TGGCGGACAT GTACGGCGGG GGCCAGGACG ACTAGGACTC 1500
CCTAAACGCC GGGCTGCAGC AGCGTCTCCA AGGGTCACT ATCCCCACGT TGGCCAAGGA 1560
CTTTGCAGCT TGTGAGAAAT TGGCCTTAGC AACTTGGAGG GAAGAGGCCT CGAAACTGAC 1620
CTCAAAGGGG CAGGTCTCTA TGCCTTTCAG AACGGAGGAA CGTGGGCAGT TTGATTTCAA 1680
CAGTGAGCAC CTCTTAGCCT AAGCCAGGGC TGCTCAATTT CTGGGAGTCT CCTCGCTACC 1740
ATAAAATGCT CAGCGCTGGG TCCCTGGGTTT TGA CTGACTC TGACTTTCCC ATGATGGCTT 1800
TTGCTCTGGA ATGGACCCCT CTCCTTAGTA ACAGGCCCTCT TACCACAATC TTCGTTTITT 1860
TTTTTTTAAAT GCTGTTTCA AAAAGTGAGA GGCAGGTCCT CAACCACCCC CTGGAGCGCT 1920
CCAGAAGCCC AGGCGTGCCC TCATGCATTT CTCTGTGGTC TCTTGGCCCC CAGACCTCCT 1980
GTTTGATTGG ATAACTGCAT TTTTATACTG AGCACGTCTA AGTGGTCCTT TATTTTAT 2040
TTTCCCTATC GAGTGCTGTA GATGAAGAGT GATGACAATC CTGTAATGT ACTAGAACTT 2100
TTTTATTAAA GGAACCTTTT CCCAAAAAAA AAAAAAAA AAAAAA 2156

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FIG. 2c.

FIG. 3a.

cDNA sequence for MDCK E-cadherin

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CGGGCACCTG TGATTCGGG AAGTCCTGCC GCCTCGCGCC GCCTCGCGCC CCGCTCTCGA   60
CCCCCGCCCG CCATGGGCC TCGGTACGGC GCGGCCCCCG CGCTCCTGCT CCCGCTGCTG   120
CTGCTGCTGC AGGTCTCATC GGGGCTCTGC CAAGAGCCGG AGCCCTGCCG CCTGGGCTTT   180
GGCGCTGACA GCTACACGTT CACCGTGCCC CGGCGACACT TGGAGAGAGG CCGTGTCCCTG   240
GGCAGGGTGA GTTTTGAAGG ATGCACCGGT CTACCCTAGGA CAGCCTATGT TTCTGATGAC   300
ACCCGATTCA AAGTGGGCAC AGATGGTGTG ATTACAGTCA AGCGGCCTCT ACAACTTCAT   360
AAACCAGAGA TAAGTTTCT TGTCCATGCC TGGGACTCCA GCCGCAGGAA GCTCTCCACC   420
AGAGTTAGGC TGAAGGCAGC GACGCACCAC CACCACCACC ATCATGATGC TCCCTCTAAA   480
ACCCAGACAG AGGTGCTCAC ATTTCCCAGT TCCCAGCATG GACTCAGAAG ACAGAAGAGA   540
GACTGGGTTA TCCCTCCTAT CAGCTGCCCCG GAAAACGAGA AAGGCCCATT TCCTAAAAC   600
CTGGTTCAGA TCAAGTCTAA CAGGGACAAA GAAATCAAGG TTTTCTACAG CATCACTGGC   660
CAAGGAGCTG ACCCACCTCC TGTGGTGTG TTTATTATTG AAAGAGAAAC AGGATGGCTG   720
AAGGTGACTG AGCCTCTGGA TAGAGAACAA ATTGCTAAGT ACATTCTCTA CTCTCATGCC   780
GTATCTTCTA ATGGGAATGC GGTGAAGAC CCAATGGAGA TCGTGATCAC GGTGACAGAT   840
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FIG. 3b.

CAGAAATGACA	ACAAGCCCGA	GTTCAACCCAG	GCAGTCTTCC	AAGGATCTGT	CACGGAAGGT	900
GCCCTTCCAG	GCACCTCTGT	GATGCAGGTG	ACAGCCACAG	ATGCGGATGA	TGATGTGAAT	960
ACCTACAACG	CTGCCATCGC	TTACAGCATC	CTCACACAAG	ACCCCTCCT	GCCTAGCAGC	1020
ATGATGTTCA	CTATCAACAA	GGACACAGGA	GTCATCAGCG	TGCTCACCAC	TGGGCTGGAC	1080
CGAGAGGGTG	TCCCCATGTA	CACCTTGGTG	GTTCAGGGTG	CTGACCTGCA	AGGCGAAGGC	1140
TTAACTACAA	CTGCAACAGC	TGTGATCACA	GTCACCTGACA	TCAATGATAA	CCCCCCCATC	1200
TTCAACCCAA	CCACGTACCA	GGGACGGGTG	CCTGAGAACA	AGGCTAACGT	CGAAATCGCT	1260
GTA CTCAAAG	TGACGGATGC	TGATGTCCCC	GATACCCCGG	CCTGGAGGGC	TGTGTACACC	1320
ATATTGAACA	ATAACAATGA	TCAATTGTGT	GTCACCACAG	ACCCAGTAAC	TAAACGACGC	1380
ATTTTGAAAA	CAACTAAGGG	CTTGGATTTT	GAGGACAAGC	AGCAGTATGT	CTTGTACGTG	1440
ACTGTGGTGA	ACGTGACCCC	GTTTGAGGTC	ATCCTCTCCA	CCTCCACAGC	CACGTCTACT	1500
GTGGACGTGG	AAGATGTGAA	TGAAGCCCCC	ATCTTCATCC	CTTGCCCCAA	GGTAGTGTC	1560
ATCCCTGAAG	ACTTTGGTGT	GGGCCAGGAA	ATCACATCCT	ACACCGCCGA	GGATCCAGAT	1620
ACATATATGG	AACAGAGGAT	AACGTATCGG	ATTGAGGG	ATGCTGCCGG	TGCGCTGGAG	1680
GTTAATCCAG	AATCTGGTGC	CATTTTCACT	CGGGCTGAGC	TGGACAGAGA	GGATTTTGAG	1740

9/42

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FIG. 3c.

CACGTGAAGA ATAGCACGTA TGAAGCCCTC ATTATAGCCA TTGACTTCGG TTCTCCAGTT 1800
GCTACTGGAA CGGGAACTCT TCTACTGGTC CTCTCTGATG TGAATGACAA TGGCCCCATT 1860
CCAGAACCTC GAAATATGGA CTTCTGCCAG AAAAACCAC AGCCTCATGT CATCAACATC 1920
ATTGATCCAG ATCTTCCCC CAACACATCT CCCTTCACAG CAGAACTAAC ACACGGCGCA 1980
AGTGTCAACT GGACCATCGA GTACAATGAC CCAGCTCGTG AATCTCTAAT TTTGAAGCCA 2040
AAGAAAACCT TAGAGTTGG TGAATAAAA ATAAATCTCA AGCTCACAGA TAACCAGAAC 2100
AAGGACCAGG TGACCACCCT ATATGTGTTT GTGTGCGACT GCGAAGGTGT CGTCAACAGC 2160
TGCAAGAGGA CGGCGCCTTA CGCCGAAGCA GGCTGTCAGG TTCCTGCCAT CTTGGGCATT 2220
CTCGGAGGAA TCCTCGCTCT ACTAATCCTG ATTCTGCTGC TTCTGCTATT TGTTCGGAGG 2280
AGAAGGTGG TCAAAGAGCC CTTACTTCCC CCAGAAGATG ACACCCGGA CAATGTTTAT 2340
TACTATGATG AAGAAGGAGG TGGAGAGGAG GATCAGGACT TTGACTTGAG CCAGTTGCAC 2400
AGGGCCTGG ATGCTCGGCC TGAAGTGAAT CGCAATGATG TGGCCCCAAC CCTCCTGAGT 2460
GTGCCCCAGT ATCGGCCCCG CCCTGCCAAT CCTGATGAAA TTGGAAACTT TATTGATGAA 2520
AACCTGAAGG CAGCGGACAC TGACCCTACT GCTCCTCCTT ATGACTCTCT GCTCGTGTTC 2580
GACTATGAAG GAAGCGGTTT TGAAGCTGCT AGTCTGAGCT CCTTGAACCT CTCAGAGTCA 2640
GACCAAGACC AGGACTATGA CTACCTGAAT GAATGGGGA ATCGCTTCAA GAAGCTGGCG 2700

10/42

SUBSTITUTE SHEET

FIG. 3d.

GACATGTATG GAGGTGGCGA GGACGACTAG GGGACTTGAG ACAAATGAAG ATGAGTCCTT 2760
ATACCATGTG GTAGAAAATG CGGAGGTGAC TGTTTTCAGC TCCCTTCATC TGAGAGGAAT 2820
TTCTGGAGAA GAGAAAATGC ACAGTGATAT ATAGTTAGGA TAGTTAGGAT TTCTACTTTA 2880
TAGATCTAAT CTGTGTGTTT GTTAGAACCA TTTTGACCCTA TTCTTTGAAG CTTTTTTTC 2940
TTTCTTTTCAT CATTCCTTTAA ATGGTGATGC TGTCCAAAAG ACCCCCCACA TGTTTATATT 3000
TCAAAAAGAAAT AGCTAAAGCC TCCAGAAAGGT TCTGCTAGCA ATTTCCGAGAT TGCCCTTATTG 3060
ACTTGTCTCA TTTTTTTAA GGAAGGTAGG GCTAAACTAC CCTATTGTGT TTGTGTGTGT 3120
GTGTGTGTAT GTGTAATTAT TTTTAAATTG TGTTCCTTTT TCTCCTATCA CTGCACCTGGT 3180
GTCCCGTGTT CTAATAACCA CTCTTAACTC CTTCTGAACT TACATTGCCCT CAGACAGGAG 3240
TTCCTCTGCTG CAGAAATTAT TGGGCCCTTT CAGGATAAGA GACTTGGTCT TAGTTTGATG 3300
GTAGTGTGAC TGGGTATTAT GGACTCGTAA GGACTTTAGT GGTCTCCTT TTTTATTTC 3360
TAAGTACATA AATTGAAATT CATATCCATC CACTGACTTG TTCTGCATTA AGTGTGTTG 3420
TCATGTGGAC GTCATTTATTG GGCTACTTTG GTTCTGAACA AGGAGCATTG ACCAGAAAAG 3480
GTGGTGAATT TTCAGGTGCC ACTCAACTTC TAATGTTTAC TTATCACTCA AACAGAAAGAG 3540
TGATCTATTC TGACGTTTAG CGTAGTGCCT GCAGTGTCTGC AGCCAAAGAT TGAAGGCGGA 3600

11/42

SUBSTITUTE SHEET

12/42

TTGTCAAAGC CAAGGGCAAC ATGAAAAATG GACTTGGAGG TGGCAGGCGG GATGGGTCAT 3660
TGAGCCCTGGC GTTTTAGCAA ACTGATGCTG AGGATAACTG AGGTGGCTCT ACCTCTAGTC 3720
CTGAAAATTC TGAAGAAATGG AAGAATCCCG ACAAAGTGTGT CCTATCGCGA TCCTTAGGTC 3780
ACAGTTTGTA CCTGAGGCCA AGAATCCCCA GGTGCCCTGCT TTTGTTAATG TCTACCGAAA 3840
ATGCAGCCCTG ATCTGGACTC AGGTGCCCCA ATTCTAAGTG TGCATAGAAA ACTGACAATA 3900
TTAGGAAATT CTTTTTCCCC CCTTAGGAGC AGGAAGAAAA TATGACCCCTA AAGGGTTTG 3960
GCAAAGGGAA GGTGGGGAGA GCTTTGACTT GGATTTTTTT TAAATTGAAA TGTGAACTTC 4020
AAGGAACTTT TGACAACCAT GGGAAATAAT TTTATCTTAA ATTGCTTTAC TGTCTGTCAG 4080
CTGTTTTTCA AAGAAAAAA AAAATCATCCC TGCAATCACT TCTTGGAATT GTCTTGATTT 4140
TTCAGCAATT TAAACTCTAA TTTAGTCCTG TATAGAGAAT GTTAATGTAG TTTTGAGTGT 4200
ATATGTGTGT GGTACGGAT AATTTGTAT TTTCTTTAGG TCTGGAAAA GAAAACAATT 4260
TAAGCTGCGA AAATTCTTAA ATATTCATTT TTATAAATTT TATTAAGAA TTTTGTATAA 4320
AAAAAAAAAA AAA 4333

FIG. 3e.

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FIG. 4b.

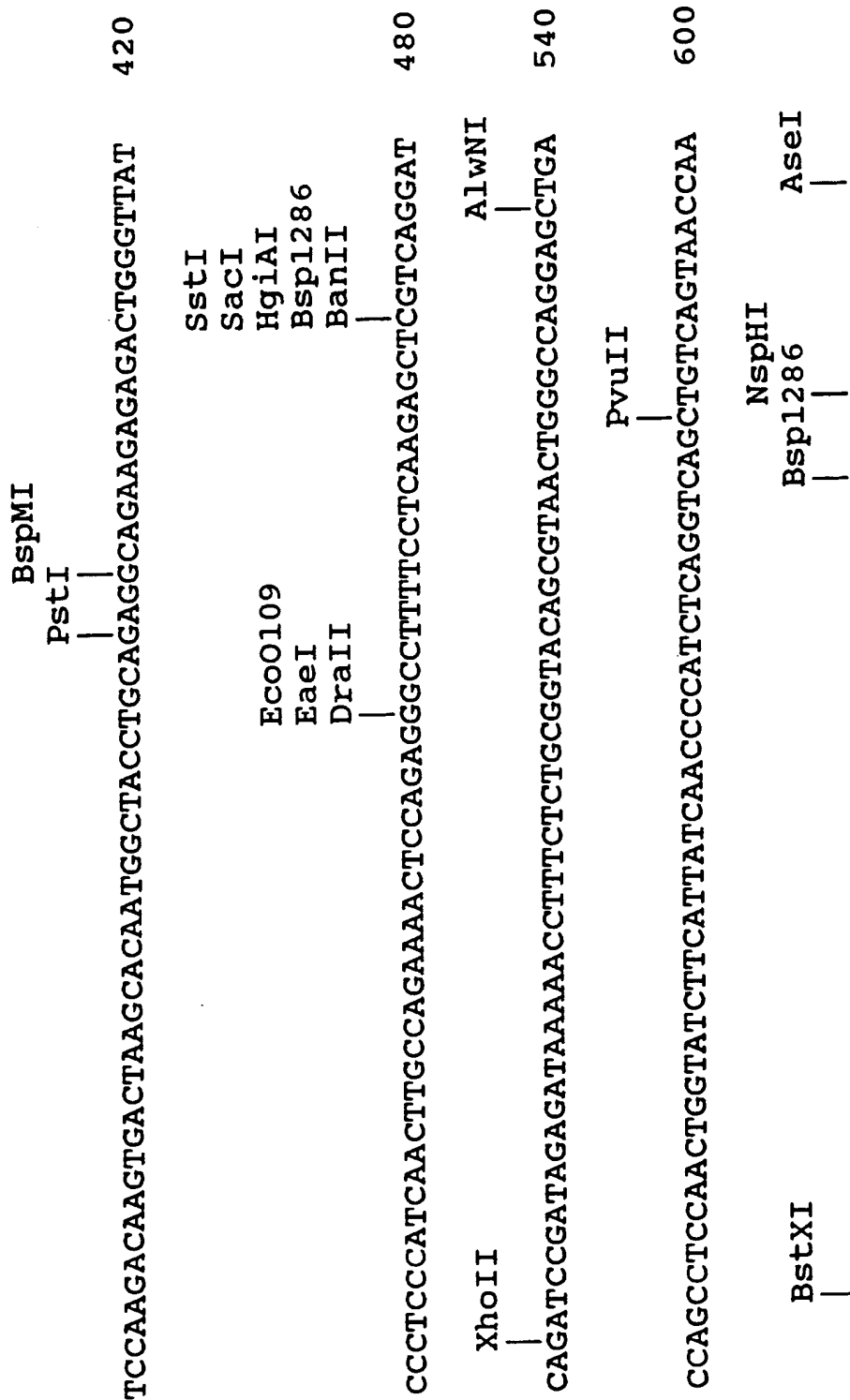


FIG. 4c.

15/42

GCCTCTGGATCGTGAGCTGATAGCCCGGTTTCATTTGAGGGCACATGCAGTGGATATTAA 660

Tth111I

TGGAACCAAGTGGAGAACCCCATCGACATTGTCTCATCAACGTTATTGACATGAATGATAA 720

SauI
Eco81I
Bsu36I
AlwNI

CAGACCTGAGTTCTTACACCAGGTTTGGAATGGGACAGTTCCTGAGGGATCAAAGCCGGG 780

NdeI

AACATATGTGATGACGGTCACTGCCGATTGATGCTGACGATCCAATGCCCTCAATGGGAT 840

HaeII
BbeI

NarI

BanI

EcoNI AhaII

NspHI

AflIII

GTTGAGGTACAGAATCCTGTCCCAGGCGCCCAAGCACCCCTTCGCCCAACATGTTTACAAT 900

PvuII

CAACAATGAGACTGGGGACATTATCACGGTGGCAGCTGGACTTGACAGAGAAAAGTACA 960

SUBSTITUTE SHEET

FIG. 4d.

16/42

ACCI		NdeI	1020
ACAGTATACGTTAATAATTCAAGCTACAGACATGGAAGGCAATCCCACATATGGCCTTTC			
	HincII	BspMII	1080
		ACCI	
CAACACAGCCACGGCTGTTCATCACGGTGACAGATGTCAACGACAATCCTCCGGAGTTAC			
TGCCATGACGTTCTATGGTGAAGTCCCTGAAACAGGGTAGATGTCAATCGTCGCTAATCT			1140
	Cfr10I		1200
AACAGTGACAGATAAGGATCAGCCCCACACACCGGCTGGACGCCCATCTACAGAATCAG			
	NaeI		1260
	Eco52I		
	EagI		
	Cfr10I		
CGGTGGAGACCCCGCGCGCTTTGGCCATTCAAACTGACCCCAACAGCAACGAGGTTT			
AGTCACCGTAGTAAACCAATCGACTTTGAAAACAAATAGGATGTATGTCTTACTGTCCG			1320
	PstI	StyI	1380
TGCAGAAAATCAAGTGCCATTAGCCAAGGGTATTTCAGCATCCACCTCAGTCAACTGCGAC		HincII	

FIG. 4e.

Tth111I ClaI
|
TGTGCTGTCACAGTTATCGATGTGAATGAAAATCCTTATTTGCCCCAAATCCAAAGAT 1440

XmnI BlnI
StuI Asp718 HpaI EcoO109
EaeI Cfr10I KpnI HincII DraII
| | |
CATTCGCCAAGAAGGCCTTCACGCCGGTACCGTGTTAAACAACGTTACTGCTCAGGA 1500

ClaI
|
CCCAGATCGATATATGCAGCAAAATATCAGATACACCAAAATTATCCGATCCTGCAAACTG 1560

GCTAAAAATAGACTCTGTGAATGGGCAGATAAATACTACCATTCGCTGTTTGGACAGAGAATC 1620

ACCGAATGTGAAAGCCCAATATATACAATGCTACTTTCCTTGCTTCTGACAAATGGAATCCC 1680

XhoII
PstI AseI
BglII
|
TCCTATGAGTGGAAACGGGAACACTGCAGATCTATTTACTTGATATTAATGACAAATGCCCC 1740

BspMII
AccIII
|
TCAAGTGTTACCTCAAGAGGCAGAGATTGTGAAACTCCGGACCCCAATTCAATTAAACAT 1800

18/42

PflMI
 |
 CACAGCACTTGATTATGACATTGATCCAAATGCTGGACCATTGTGCTTTTGATCTTCCTTT 1860

 CelII
 |
 GTCTCCAGTGACTATTAAAGAGAAATTGGACCATCACTCGGCTTAATGGTGATTTTGCTCA 1920

 XhoII
 |
 GCTTAACTTAAAGATAAAATTTCTTGAGGCCGGGATCTACGAAGTCCAATCATAATCAC 1980
 AGATTGGGTAAATCCTCCCAAATCGAATATCTCCATCCTTCGGGTGAAGGTTGCCAGTG 2040

 Cfr10I
 Bsp1286
 Bani Bani
 | |
 TGATTCCAACGGGACTGCACAGATGTGGATCGAATTGTGGAGCAGGGCTGGGCACCGG 2100

 HaeII
 BbeI
 NarI
 AhaII
 | |

FIG. 4g.

19/42

CGCCATCATCGCCATCCTGCTTTGGCATCATCATCCTGCTCATTTCTCGTTCTGATGTTTCGT 2160

GGTATGGATGAAACGCCGGGATAAAGAACGCCAGGCCAAACAACCTTTTAATTGATCCAGA 2220

AGATGATGTAAGAGATAATATTTTAAATATGATGAAGAGTGAGGAGAGAAGACCA 2280

GGA CTACGATTGAGCCAGCTCCAGCAGCCTGATACGGTAGAGCCAGATGCCATCAAGCC 2340

AGTTGGAATCCGACGGTTGGATGAGAGGGCCCATCCATCGGAGCCCCCAGTACCCGGTTTCG 2400

ATCTGCAGCCCCACACCCAGGGACATCGGGGACTTCATTAAATGAGGGCCCTTAAAGCTGC 2460

TGACAAAGATCCCACCGCTCCGCCCTACGACTCCCTCTTAGTCTTTGACTATGAAGGCAG 2520

TGGCTCCACGGCGGTCCTTGAGCTCCCTTAAATTCCTCCAGTAGTGAGGTGAGCAGGA 2580

DraI
SspI AhaIII

EaeI
Bsp1286
BamII

EcoO109
EaeI
AseI
DraII

PstI

SstI
SacI
HgiAI
EcoO109
Eco52I
EagI
DraII
Bsp1286
BamII

SUBSTITUTE SHEET

SUBSTITUTE SHEET

FIG. 4i.

BanII
 CCAATACTGTTTGGAACACACTGAGCTCAGTTACACTTGAATTTTACAGTACAGAAGCAC 2880
 TGGGATTTATGTGCCCTTTTGTACCTTTTTCAGATTGGAATTAGTTTATGTTTAAAGGC 2940
 SspI
 TTTAATGGTACTGATTTCTGAAATGATAAGTAAAGACAAAATATTTTGTGTGGAGCA 3000
 GTAAGTTAAACCATGATATGCTTCGACACGCTTTTGTGTACATCGCAATTGTGCTTTTATTAA 3060
 StyI
 AAATATGGAATTAAACAGACAAACCACTCATGGAGCAATTTATTACCTTGGGGC 3120
 BstXI
 TGAGACCATGAGATTGGAAATGTACATTATTCTAGTTTGTAGACTTTAGTTTCTGTTT 3180
 PvuII
 TGTTTTTTTTCCACTAAAATCTTAAACTTACGCAGCTGGTTGCAATAAAGGAGTT 3240
 XmnI
 TTCATATCACC AATTGTAGCAAAATTGAATTTTTCATAAACTAGATGTTAGACACAT 3300
 TTGGTCTTAATCCATGTACACTTTTATTATTACTGTATTTTCCACTTCACTGTAAA 3360
 ATGGTATGTACATAATGTTTATTGGCATACTATGGAGAAAGTGCAGAAACTTCAGA 3420

21/42

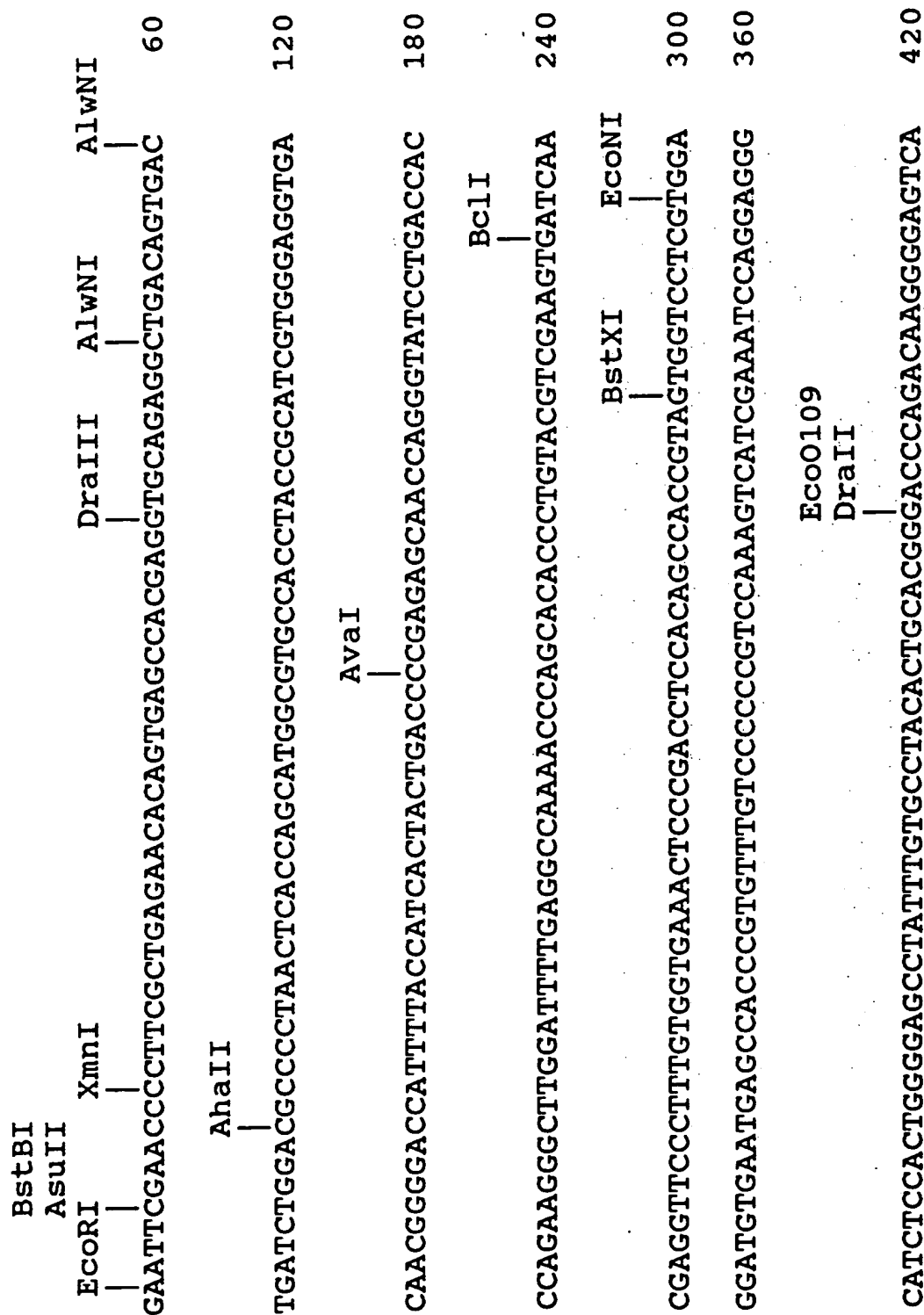
22/42

[illegible]

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FIG. 4k.

P-cadherin restriction map



23/42

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FIG. 4I.

24/42

GAAGATCAGTTACCATCCTGAGAGACCCAGCAGGGTGGCTAGCGATGGACCCAGACAG 480
 TGGACAAGTCACTGCCGCAGGGGTCTTGGACCGTGAGGATGAGCAGTTTGTGAGAAACAA 540
 CATCTACGAAGTCATGGTCTTGGCCACAGATGATGGAGCCCTCCACCACTGGCACAGG 600
 GACCCCTCCTGCTAACACTGATGGACATCAATGACCAACGGTCCCGGTCCCGAGCCCCGTCA 660
 GATCACCATCTGCAACCAAGCCCTGTGCCCCAGGTGCTAAACATCACAGACAAGGACTT 720
 GTCCCCCACA CTGCCCCCTTTCCAGGCCCAACTCACACATGACTCGGACGTCCTATTGGAC 780
 AGCAGAAGTCAACGAGAAAGGAGACGCAGTAGCCCTTGTCCCTGAAGAAAGTTCTCTAAAGCA 840

Restriction sites indicated by vertical lines above the sequence:
 NheI (between 480 and 540)
 BstXI (between 480 and 540)
 PflMI (between 480 and 540)
 Bsp1286 (between 540 and 600)
 BalI (between 600 and 660)
 Eco0109 (between 660 and 720)
 DraII (between 660 and 720)
 Bsp1286 (between 720 and 780)
 EaeI (between 780 and 840)
 HincII (between 840 and 900)
 AatII (between 780 and 840)
 AhaII (between 780 and 840)
 XmnI (between 840 and 900)

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FIG. 4m.

25/42

HgiAI		PvuII		900
Bsp1286				
ApaI				
AGGCGAATACGATGTGCACCTTTCCCTGTCCGACCACGGCAACAAGGAACAGCTGACAGT				
BclI	DraIII	BspMI	EcoO109	
			DraII	
				960
GATCAGAGCCACCGTGTGTGACTGCCACGGCAACATGGTGACCTGCCGGGACCCCTGGAC				
GTGGGGTTTCCTCCTCCCATCCTGGGTGCTGCCCTGGCTCTGCTGCTCCTTCTGCTGCT				
HgiAI		XmnI		1080
Bsp1286				
GCTCCTATTCTTGGTGAGAAAGAAACGGAAGATCAAGGAACCCCTTCTCCTCCCAGAAGA				
Tth111I				1140
TGATACCGTGACAACGTCTTCTACTACGGCGAAGAGGGGGTGGCGAGGAGGACCAGGA				
		SauI		
		Eco81I		
		Bsu36I		
CTATGACATCACCAGCTCCACCGGGTCTGGAGGCCCGCCCTGAGGTGGTCTCCGCAA				
				1200

FIG. 4n.

26/42

BanI			
	CGATGTGGCACCATCCTTTCATCCCCACACCCATGTACCGTCTCTGGCCAGCCAAACCCAGA	1260	
	TGAAATCGGCAACTTCATCATTTGAGAACTGAAGGCAGCCAAACACAGACCCACGGCCCC	1320	
	GGCCTACGACTCCCTGTGGTGTTCGACTATGAGGGCAGTGGCTCCGATGCCGCTCTCT	1380	
SstI			
SacI			
HgiAI			
Bsp1286			
BanII			
	GAGCTCGCTCACCTCCTCAACCTCTGACCAGGACCAAGACTACAACTATCTGAATGAGTG	1440	
	GGGCAGCCGCTTCAAGAAGCTGGCGGACATGTACGGCGGGGCCAGGACGACTAGGACTC	1500	
	CCTAAACGCCGGCTGCAGCAGCGTCTCCAAGGGTCACTATCCCCACGTTGGCCCAAGGA	1560	
	CTTTGCAGCTTGTGAGAAATTGGCCCTTAGCAACTTGGAGGGAAGAGGCCCTCGAAACTGAC	1620	

FIG. 4o.

27/42

BspMI
 |
 CTCAAAGGCGAGGTCTCTATGCCCTTTCAGAAACGAGGAAACGTGGGCAGTTTGATTTCAA 1680

 HgiAI
 Bsp1286 EcoNI
 |
 CAGTGAGCACCTCTTAGCCTAAGCCAGGGCTGCTCAATTCTCTGGGAGTCTCCTCGCTACC 1740

 EcoO109
 DraII
 Eco47III
 CelII HaeII
 | | | |
 ATAAAAATGCTCAGCGCTGGGTCCCTGGGTTTGTGACTGACTCTGACTTCCCATGATGGCTT 1800

 StuI
 EaeI
 | |
 TTGCTCTGGAATGGACCCCTTCTCCTTAGTAACAGGCCCTCTTACCACAATCTTCGTTTTT 1860

 EcoO109 HaeII
 BspMI DraII PflMI Eco47III
 | | | |
 TTTTTTTAATGCTGTTTTTCAAAAAGTGAGAGGCAGGTCTCAACCACCCCTGGAGCGCT 1920

 Bsp1286 NsiI
 |
 CCAGAAGCCAGCGGTGCCCTCATGCATTTCTCTGTGCTCTCTTGGCCCCCAGACCTCCT 1980

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FIG. 4p.

HgiAI
Bsp1286

GTTTGATTGGATAACTGCAATTTTATACTGAGCACGCTAAGTGGTCCTTTATTTTAT 2040

TTTCCCTATCGAGTGTAGATGAAGAGTGATGACCAATCCTGTAAATGTACTAGAACTT 2100

Xmnl

TTTATTAAAGGAACCTTTTCCCAAAAAAAAAAAAAAAAAAAC

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E-cadherin restriction map

Bani

CGGGCACCTGTGATTCCGGGAAGTCTGCCGCTCGCGCCGCTCGCGCGCTCTCGA 60

BanII
ApaI

HaeII
BbeI

EaeI **NarI**
StyI **Eco0109** **AhaII**
NcoI **DraII** **BanI**

NarI
AhaII
BamI

CCCCCGCCGCATGGGCCCTCGGTACGGGGGCCCGCGCTCCTGCTCCCGCTGCTG 120

BspMI **PstI**

BanII

BanII

Bq11

Bq11

CTGCTGCTCAGGTCATCGGGGCTCTGCCAAGAGCCGGAGCCCTGCCGCCCTGGCTTT

FIG. 4q.

[illegible]

29/42

SUBSTITUTE SHEET

StyI

FIG. 4r.

CAAGGAGCTGACGCACCTCCTGTTGGTGTGTTTATTATTGAAAGAGAAACAGGATGGCTG 720

AAGGTGACTGAGCCTCTGGATAGAGAAACAAATTGCTAAGTACATTCTCTACTCTCATGCC 780

GTATCTTCTAATGGGAATGCGGTTGAAGACCCCAATGGAGATCGTGATCACGGTGACAGAT 840

CAGAATGACAACAAGCCCGAGTTCACCCAGGCGAGTCTTCCAAGGATCTGTGTACGGAAGGT 900

GCCCTTCCAGGCACCTCTGTGATGCAGGTGACAGCCACAGATGCGGATGATGTGAAT 960

ACCTACAACGCTGCCATCGCTTACAGCATCCTTCACACAAGACCCCTCCTGCCCTAGCAGC 1020

ATGATGTTCACTATCAACAAGGACACAGGAGTCATCAGCGTGCTCACCACTGGGCTGGAC 1080

CGAGAGGGTGTCCTCCCATGTACACCTTGGTGGTTTCAGGCTGCTGACCTGCAAGGCCAAGGC 1140

BsmI

BclI

AvaI

StyI

BanI

XhoII

BanI BspMI

HgiAI

BstXI

StyI

BspMI

SUBSTITUTE SHEET

FIG. 4s.

31/42

<p>BclI PvuII</p>	<p>AlwNI</p>	
TTAACTACAAGTGAACAGCTGTGATCAGCTGATCAGTCAATGATAACCCCCCATC 1200		
<p>BanI</p>		
TTCAACCCAAACGTACCGGACGGGTGCTGAGAACAGGCTAACGTCGAAATCGCT 1260		
	<p>BglI</p>	
GTACTCAAAGTGACGGATGCTGATGTCCCCGATACCCCGGCTGGAGGGCTGTGTACACC 1320		
<p>BclI</p>		
ATATTGAACAATAACAATGATCAATTTGTGTCAACACAGACCCAGTAACTAACGACGGC 1380		
	<p>AlwNI</p>	
ATTTTGAAAACAATAAGGGCTTGGAATTTTGAGGACAAAGCAGCAGTATGTCTTGTACGTG 1440		
	<p>AlwNI</p>	
ACTGTGGTGAACGTGACCCCGTTTGAGGTCACTCCTCTCCACCTCCACAGCCACTGTCACT 1500		
GTGGACGTGGAAGATGTGAATGAAGCCCCCATCTTCATCCCTTGCCCCAAAGGTAGTGTC 1560		
	<p>XhoII BamHI</p>	
ATCCCTGAAGACTTTGGTGTGGGCCAGGAAATCACATCCTACACCGCCGAGGATCCAGAT 1620		
	<p>Cfr10I</p>	

32/42

FIG. 4t.

ACATATATGGAACAGAGGATAACGTATCGGATTTGGAGGGATGCTGCCGGTTGGCTGGAG 1680

BanI
PflMI
AlwNI
AvaI CellII

GTTAATCCAGAATCTGGTGCCATTTTCACTCGGGCTGAGCTGGACAGAGGATTTTGAG 1740

HgiAI

CACGTGAAGAAATAGCACGTATGAAGCCCTCATATAGCCATTGACTTCGGTTCCTCCAGTT 1800

GCTACTGGAACGGGAACCTTCTACTGGTCCCTCTCTGATGTGAATGACAATGGCCCCATT 1860

CCAGAACCTCGAAATATGGACTTCTGCCAGAAAAACCCACAGCCTCATGTCAACAACATC 1920

XhoII
BglII

ATTGATCCAGATCTTCCCCCAACACATCTCCCTTCACAGCAGAACTAACACACGGCGCA 1980

HincII

AGTGTCAACTGGACCATCGAGTACAATGACCCAGCTCGTGAACTCTTAATTTGAAGCCA 2040

AAGAAAACTTTAGAGTTGGGTGACTACAAAATAAATCTCAAGCTCACAGATAACCAGAAC 2100

BstEII

PvuII

HincII

AAGGACCAGGTACCACCCCTATATGTGTTTGTGTGCGACTGCCGAAGGTGTCGTCAACAGC 2160

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SUBSTITUTE SHEET

37/42

DraI
AhaIII
|
GCAAAGGAAGGTGGGAGAGCCTTGGACTTGGATTTTTTTTAAATTGAAATGTGAACCTC 4020

StyI
NcoI
|
AAGGAACCTTTTGACAACCATGGGAAATAATTTTATCTTAAATTGCTTTACTGTCTGTCAG 4080

PvuII
|
CTGTTTTTCAAGAAAAAAATCATCCCTGCAATCACTTCTTGGAAATGTCTTGATTT 4140

DraI
AhaIII
|
TTCAGCAATTAAACTCTAATTAGTCCTGTATAGAGAATGTTAATGTAGTTTTCAGTGT 4200

ATATGTGTGGGTACGGATAATTTTGTAATTTCTTTAGGCTCGAAAGGAAACAATT 4260

SspI
|
TAAGCTGCGAAAAATTCTTAAATATTCATTTTTTATAAATTTTATTAAAGAAATTTGTAAA 4320

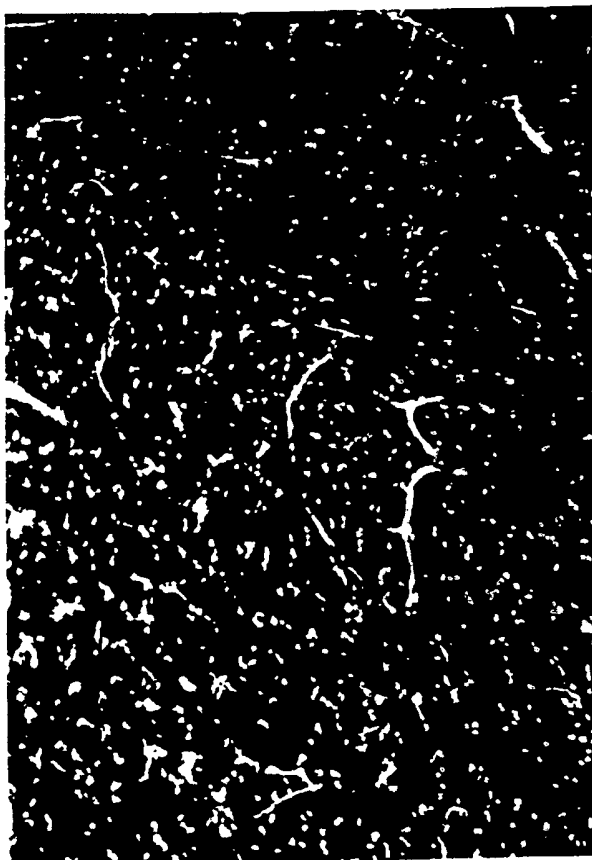
AAAAAAAAAAAAA 4333

FIG. 4y.

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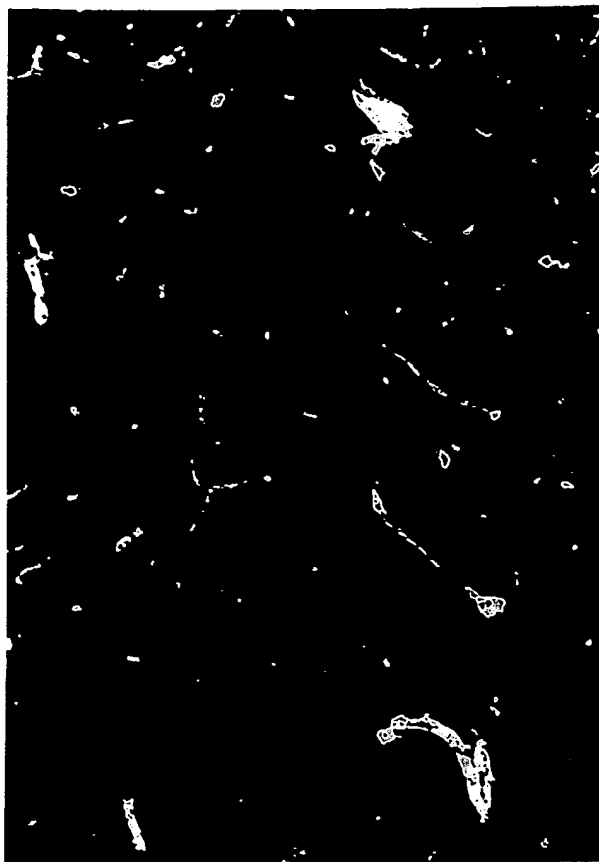
38/42

FIG. 5.



39/42

FIG. 6.



40/42

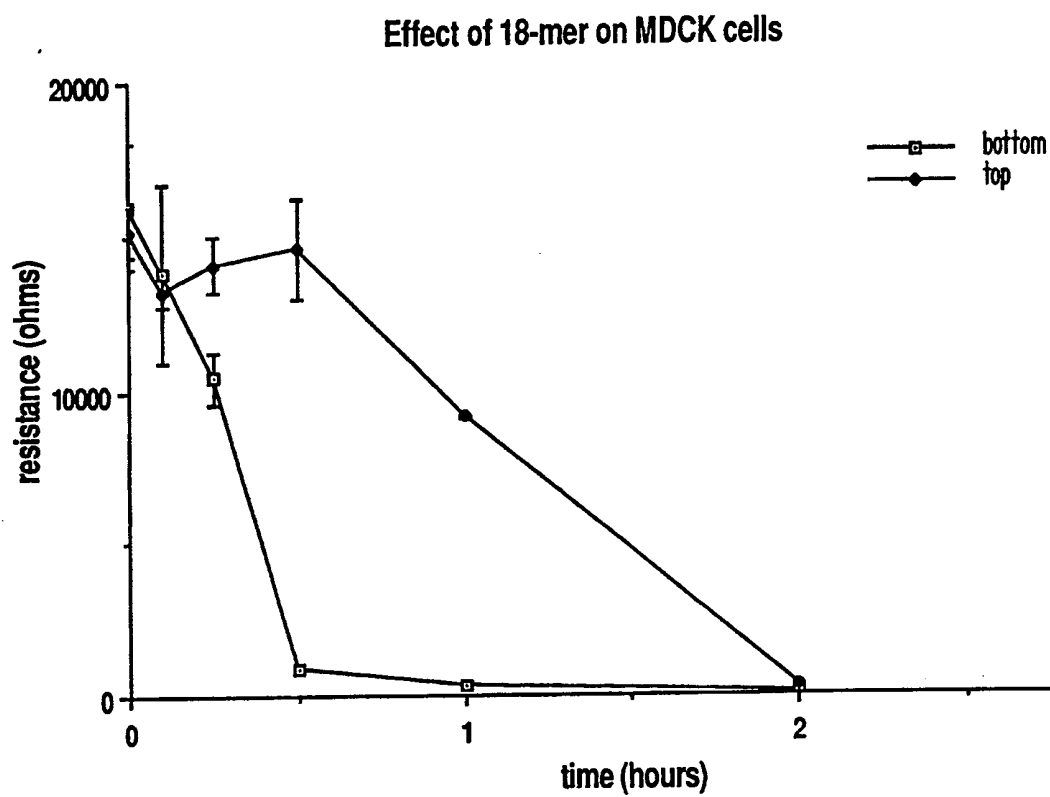


FIG. 7.

41/42

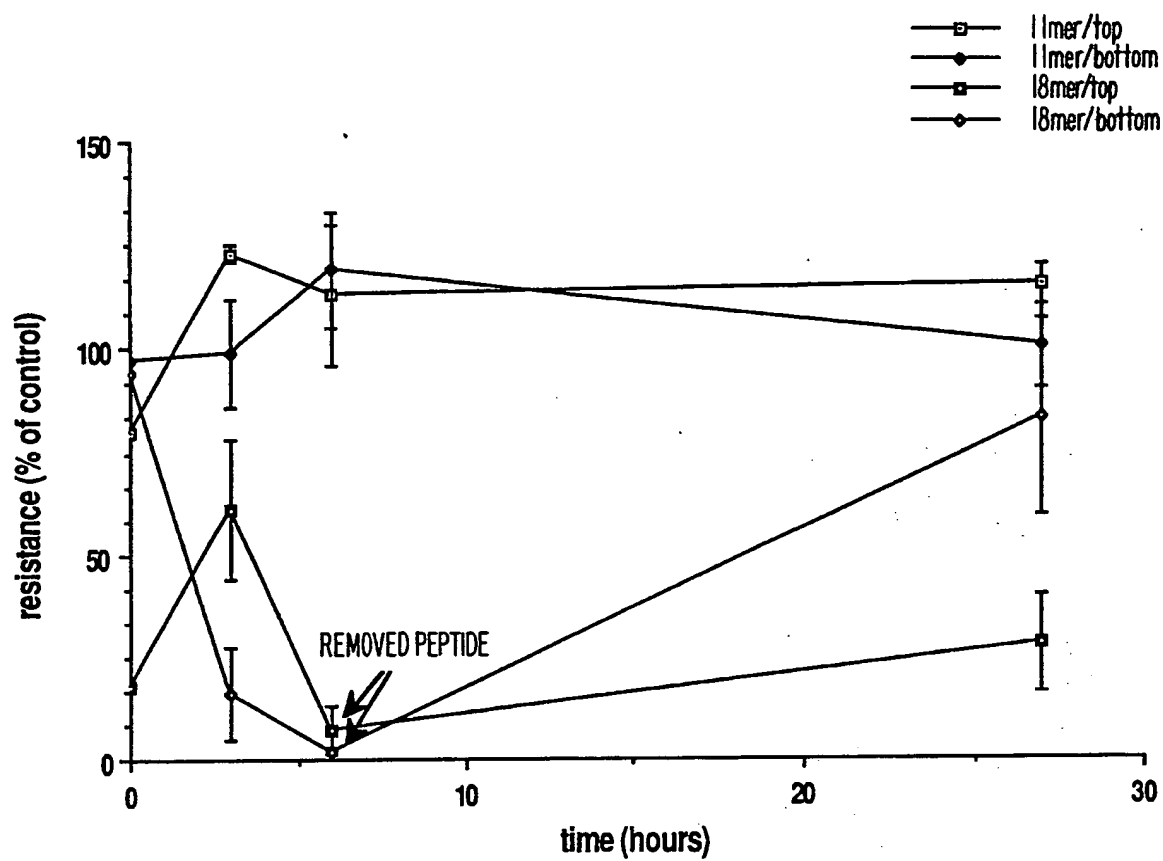


FIG. 8.

42/42

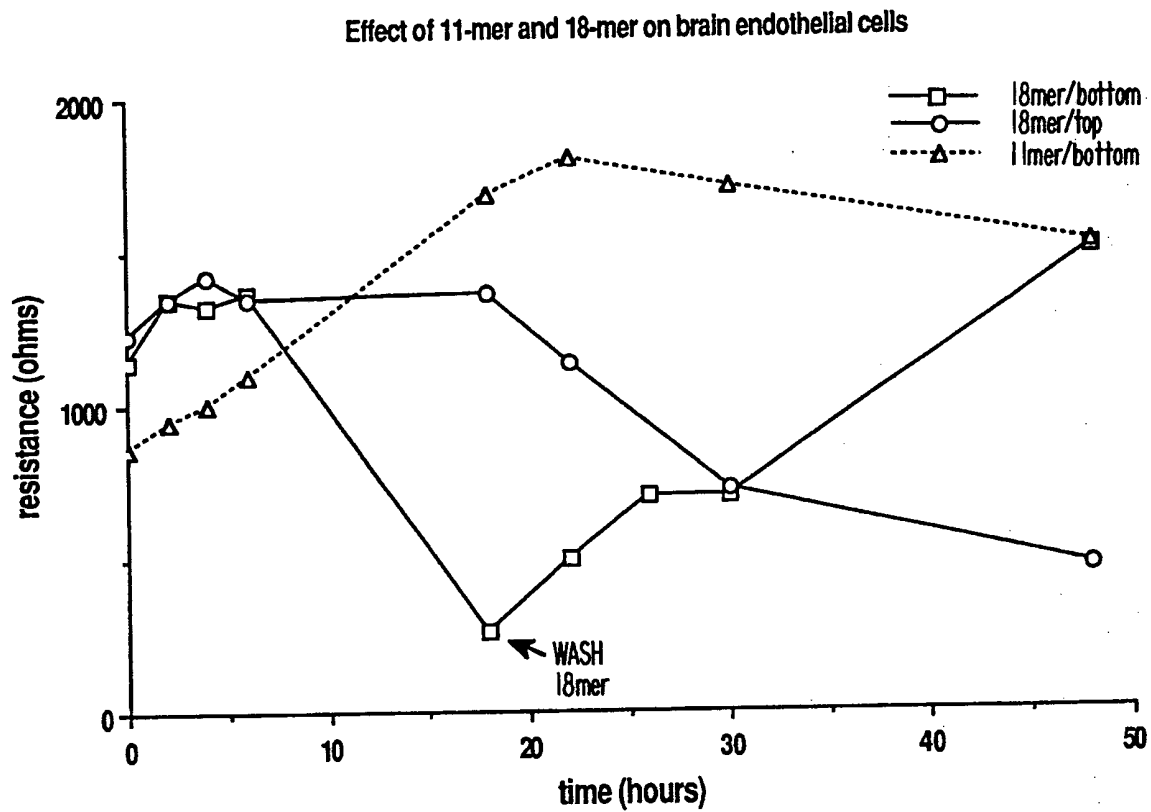


FIG. 9.

INTERNATIONAL SEARCH REPORT

International Application No PCT/US90/05105

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) ¹

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC(5): A61K 37/02, 39/00; C07K 7/08, 7/10, 13/00, 15/00, 15/28

U.S.Cl.: 530/324, 326, 350, 389, 390, 391, 402, 409, 345, 387; 514/12, 13; 424/85.8, 85.91

II. FIELDS SEARCHED

Minimum Documentation Searched ⁴

Classification System ¹

Classification Symbols

530/324, 326, 350, 389, 390, 391, 402, 409, 345, 387
514/12, 13
424/85.8, 85.91

U.S. Cl.

Documentation Searched other than Minimum Documentation
to the extent that such Documents are Included in the Fields Searched ⁵

Data bases: Dialog (Files; Medline, Biosis, Chemical Abstracts, World Patents Index) Automated Patent Searching (1975-1990)

III. DOCUMENTS CONSIDERED TO BE RELEVANT ¹¹

Category ⁶	Citation of Document, ¹² with indication, where appropriate, of the relevant passages ¹³	Relevant to Claim: No. ¹⁴
<u>X</u> Y	The EMBO Journal, Volume 4, No. 13A, issued December 1985, Vestweber et al., "Identification of a Putative Cell Adhesion Domain of Uvomorulin," pp. 3393-3398, See the Abstract and Discussion.	1-6,14-21,23-27 & 35-42 1-65
Y	Development, Volume 102, issued April 1988, M. Takeichi, "The Cadherins: Cell-cell Adhesion Molecules controlling Animal Morphogenesis," pp. 639-655 see the Summary and pages 643, 645 and 651.	1-65
<u>X</u> Y	The Journal of Cell Biology, Volume 107, issued October 1988, B. Gumbiner et al., "The Role of the Cell Adhesion Molecule Uvomorulin in the Formation and Maintenance of the Epithelial Junctional Complex," pp. 1575-1587 see the Abstract.	1-6,14-21,23-27, 35-42 1-6,14-27,35-47, 55-65

⁶ Special categories of cited documents: ¹⁵

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search ²

21 November 1990

International Searching Authority ¹

ISA/US

Date of Mailing of this International Search Report ³

04 FEB 1991

Signature of Authorized Officer ¹⁰

R. Keith Baker
R. Keith Baker, Ph.D.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, ¹⁶ with indication, where appropriate, of the relevant passages ¹⁷	Relevant to Claim No ¹⁸
Y	The EMBO Journal, Volume 6, No. 12, issued 1987, M. Ringwald et al., "The Structure of Cell Adhesion Molecule Uvomorulin Insights into the Molecular Mechanism of Ca ²⁺ -dependent Cell Adhesion," pp3347-3353, see pages 3647-3648.	1-13,22-34,43-54 and 63-65
Y	US, A, 4.671,958 (Rodwell et al.) 09 June 1987, see the Abstract and Column 7.	43-47 and 55-65
Y,P	Development Biology, Volume 139, No. 1, issued May 1990, O.W. Blaschuk et al., "Identification of a Cadherin Cell Adhesion Recognition Sequence," pp227-229, see the entire Document.	1-65

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE¹

This international search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. ☐ Claim numbers _____, because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers _____, because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out¹, specifically:

3. ☐ Claim numbers _____, because they are dependent claims not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. ☒ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING²

This International Searching Authority found multiple inventions in this international application as follows:

See Attachment

1. ☒ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application. **Telephone Practice**
2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:
3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:
4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

- ☐ The additional search fees were accompanied by applicant's protest.
☐ No protest accompanied the payment of additional search fees.

Attachment To PCT/ISA/210
Observations Where Unity Of Invention Is Lacking

Group I, claims 1-13 and 22-34, drawn to a composition for opening tight junctions and a method of use, classified in classes 530 and 514, subclasses 324, 326, 350 and 12 and 13, respectively.

Group II, claims 14-21 - 35-42, drawn to antibodies for opening tight junctions and methods of use, classified in classes 530 and 424, subclasses 387 and 85.8, respectively.

Group III, claims 43-54 and 63-65, drawn to a conjugates of a drug and a cell adhesion inhibitor, classified in class 530, subclasses 402, 409, and 345.

Group IV, claims 55-62, drawn to a conjugate of a drug and an antibody, classified in classes 530 and 424, subclasses 389, 390, 391 and 85.91, respectively.

Attachment To PCT/ISA/210

Detailed Reasons For Holding Lack Of Unity Of Invention:

PCT Rule 13.2 permits claims to "a" (one) product, "a" (one) method of making and "a" (one) method of using said product. The invention as set forth in Group I constitutes a combination of a product and a method of use. Groups II, III and IV one drawn to products that are distinct from that of Group I. Each of the products have a different structure and are distinct compositions as evidenced by their separate classification.

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